18 November 2005

Science

Vol. 310 No. 5751
Pages 1073–1228 $10

Materials *for and from* Biology

125 YEARS OF GLOBAL Science

AAAS

introducing the

Time MACHINE

the MINI PREP 96

Fully Automatic plasmid and genomic DNA purification at the push of a button.

START
ENTER

Your time is valuable.

MACCONNELL RESEARCH

800.466.7949 www.macconnell.com

If it's in there, you'll find it.

StrataScript® QPCR cDNA Synthesis Kit generates cDNA from even the smallest amounts of RNA quickly and reliably.

Our StrataScript® Quantitative PCR (QPCR) cDNA Synthesis Kit* delivers maximum RNA sensitivity and dynamic range down to sub-picogram RNA levels. Achieve high efficiency reverse transcription and better R Squared values in our new buffer system and master mix format, which greatly reduces sample transfer errors known to hamper results. Each batch is QPCR-qualified to ensure you produce the most reliable two-step quantitative reverse-transcriptase PCR (QRT-PCR) data.

- **Maximum RNA sensitivity**
- **Excellent linearity**
- **Most reliable two-step QRT-PCR data**

Need More Information? Give Us A Call:

Stratagene USA and Canada
Order: (800) 424-5444 x3
Technical Services: (800) 894-1304 x2

Stratagene Europe
Order: 00800-7000-7000
Technical Services: 00800-7400-7400

Stratagene Japan K.K.
Order: 03-5159-2060
Technical Services: 03-5159-2070

www.stratagene.com

Ask Us About These Great Products:

StrataScript® QPCR cDNA Synthesis Kit	**50 rxn**	**600554**

*Purchase of this PCR-related product does not convey any rights under the foreign counterparts of the PCR patents owned by Roche Molecular Systems. A license to use the PCR process, where such process is covered by patents, accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

STRATAGENE®

GE Healthcare

Why do 100,000 scientists trust GE Healthcare for all their protein purification needs?

Here's Ä clue.

To 100,000 scientists worldwide, the name ÄKTA™ has always meant outstanding protein purification, and now it's brought to you by GE Healthcare. With the ability to purify virtually 100% of all biomolecules, the ÄKTAdesign™ platform can handle the toughest of challenges. Whatever the scale, from laboratory, to process development and manufacturing, there's an ÄKTAdesign system to meet every need. All systems in the ÄKTAdesign family work with the intelligent UNICORN™ software, which makes it easy to control every stage of your purification processes. Accurate, reproducible results just take a little pure imagination.

Visit www.amershambiosciences.com/aktadesign

imagination at work

GE15-05

Science

Special Issue

Materials and Biology

Confocal micrograph of an artificial compound eye produced by biologically inspired optical system synthesis. Each microlens is individually self-aligned with an artificial cone and a waveguide. See page 1148. [Image: K. Jeong]

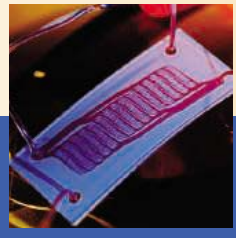
Volume 310
18 November 2005
Number 5751

Introduction

News

Reviews

Related Brevia page 1151



For related online content see page 1083 or go to www.sciencemag.org/sciext/materials/

Departments

1106

News of the Week

1125 & 1174

News Focus

Letters

Books *et al.*

Policy Forum

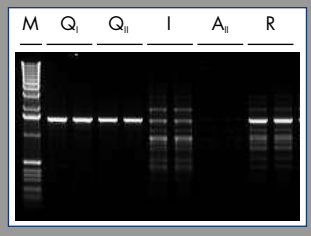
Contents continued

Systems Biology — DNA Amplification

Hit your hot-start PCR target first time — the best just got faster!

New

Gel-tracking dyes in HotStarTaq *Plus* PCR buffer enable immediate gel loading



Higher PCR specificity with QIAGEN (Q_I, Q_{II}) than with enzymes from Supplier I, A_{II}, or R

New hot-start enzymes for high PCR specificity and sensitivity with minimal optimization!

Specificity, sensitivity, and easy handling, combined with minimal optimization are the key reasons why researchers trust QIAGEN® enzymes. Now, with our 2 new hot-start PCR enzymes, you get successful results faster and more conveniently than ever before!

HotStarTaq® *Plus* DNA Polymerase

- Fast 5-minute enzyme activation time combined with unrivaled performance
- Choice of original or ready-to-load PCR buffer for added convenience

HotStar HiFidelity Polymerase Kit

- Increased sensitivity and reliability
- New high-fidelity enzyme with 10-fold higher fidelity than *Taq* DNA Polymerase

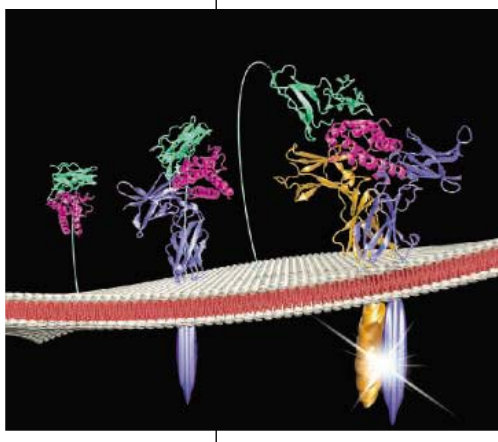
For fast and successful PCR results — visit www.qiagen.com/goto/PCR .

QIAGEN

Science

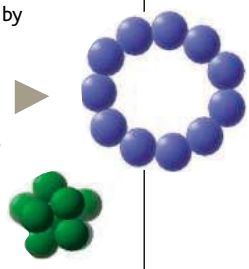
Perspectives

Science Express www.sciencexpress.org



Credits: M. Rickert & K. C. Garcia

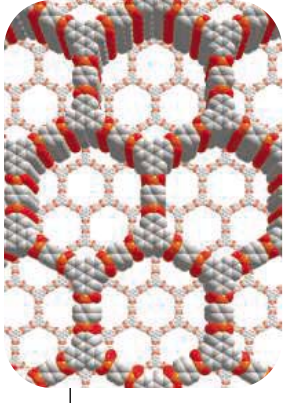
1159



Brevia

Research Articles

Reports



1166

Contents continued

What if moving from one particular protein to the most relevant journal and patent literature were as easy as pushing a button?

It is.

Not only does SciFinder provide access to more proteins and nucleic acids than any publicly available source, but they're a single click away from their referencing patents and original research.

Coverage includes everything from the U.S. National Library of Medicine's (NLM) MEDLINE® and much more. In fact, SciFinder is the only single source of patents and journals worldwide.

Once you've found relevant literature, you can use SciFinder's powerful refinement tools to focus on a specific research area, for example: biological studies such as target organisms or diseases; expression microarrays; or analytical studies such as immunoassays, fluorescence, or PCR analysis. From each reference, you can link to the electronic full text of the original paper or patent, plus use citation tools to track how the research has evolved and been applied.

Visualization tools help you understand results at a glance. You can categorize topics and substances, identify relationships between areas of study, and see areas that haven't been explored at all.

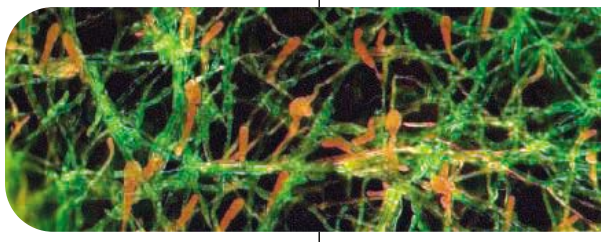
Comprehensive, intuitive, seamless—SciFinder directs you. It's part of the process. To find out more, call us at 1-800-753-4227 (North America) or 1-614-447-3700 (worldwide) or visit www.cas.org/SCIFINDER.

SciFinder®

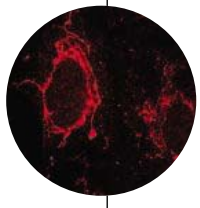
Part of the process.SM

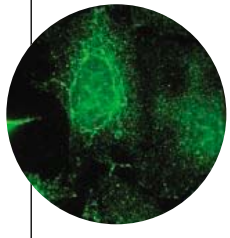
CAS®

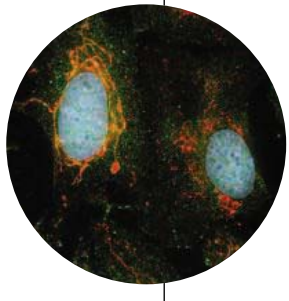
REPORTS CONTINUED



1180







1128 & 1187

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

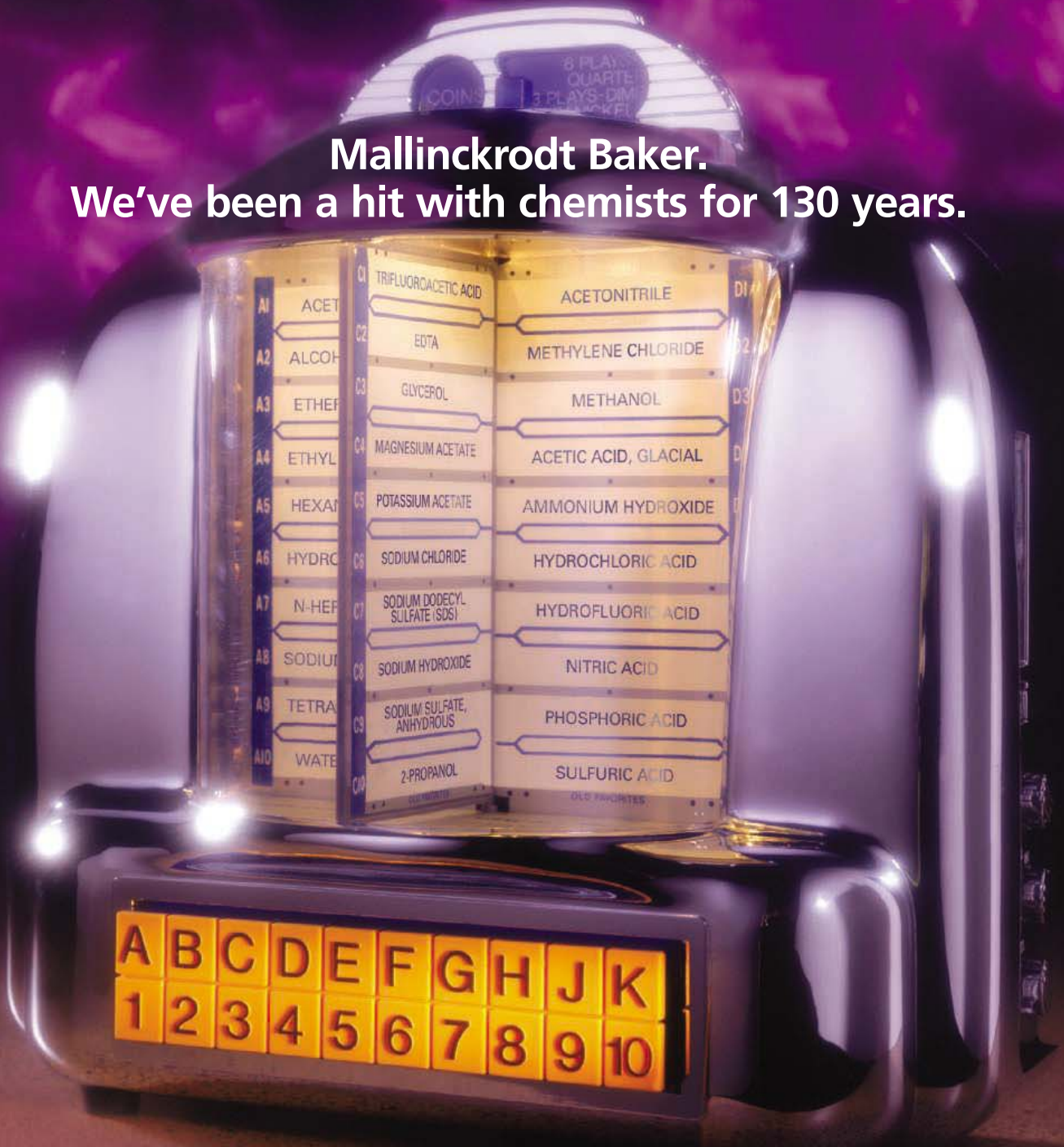
SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices.

Domestic individual membership and subscription (51 issues): $135 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $550; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. **Printed in the U.S.A.**

Change of address: allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to *Science*, P.O. Box 1811, Danbury, CT 06813–1811. **Single copy sales:** $10.00 per issue prepaid includes surface postage; bulk rates on request. **Authorization to photocopy** material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that $15.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for *Science* is 0036-8075/83 $15.00. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

Contents continued

Mallinckrodt Baker.
We've been a hit with chemists for 130 years.



J.T.Baker® and Mallinckrodt®: Two great brands. One unique source for chemicals.

Every day, thousands of laboratories around the globe put their trust in chemicals from Mallinckrodt Baker. Our diverse product line offers a match for virtually any application you're working on. Plus, our chemicals deliver consistent results…time after time.

For details about the chemicals that are right for your application, visit www.mallbaker.com/lab

Mallinckrodt Baker

J.T.Baker® Mallinckrodt® CHEMICALS

Quality • Choice • Value

sciencenow www.sciencenow.org Daily News Coverage

Flying by Feel
Touch receptors on bats' wings aid aerial pursuit of prey.

Charge of the Caterpillars
Climate change could cause an explosion in the insect's numbers.

Driven to Create?
New study suggests genetic link between bipolar disorder and artistic tendencies.

The new ScienceCareers.org

ScienceCareers.org www.sciencecareers.org Career Resources for Scientists

US: ScienceCareers.org—Your Science Career, in a Nutshell *J. Austin*
Science Magazine's two career sites, Next Wave and ScienceCareers.org, are now one.

US: Tooling Up—Developing Resilience *D. Jensen*
Making it through graduate school, and beyond, requires a thick skin.

Europe: Using Math to Predict Physical Phenomenon *A. Forde*
Applied mathematician Snorre Christiansen aims to predict how black holes might collide.

Europe: Industry Insider—The European Steel Industry, a Phoenix Rising from the Ashes *A. Michels*
The European steel industry is seeking young researchers to fill their research labs.

MiSciNet: Clinton Junior College—Monitoring, Motivating, and Mentoring Students in STEM *C. Parks*
Students are preparing for science and technology careers thanks to the Curriculum Institute Partnership Award, sponsored by NASA.

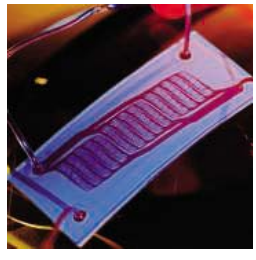
science's sage ke www.sageke.org Science of Aging Knowledge Environment

related Materials and Biology Section page 1131

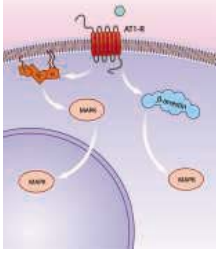
▶ **News Synthesis: Bionic Grandma** *R. J. Davenport*
Engineered tissues might someday rejuvenate the elderly.

Perspective: Immune Shaping and the Development of Alzheimer's Disease Vaccines *H. J. Federoff and W. J. Bowers*
The search is on for efficacious AD vaccines that do not promote dangerous brain inflammation.

News Focus: A Switch in Time Saves Mind *M. Leslie*
Aging rats preserve their memory by adjusting their response to stimulation.



Rejuvenating tissues.



Activating cytosolic MAPK with β-arrestin.

science's stke www.stke.org Signal Transduction Knowledge Environment

Connections Map: Angiotensin II– Stimulated Signaling Through G Proteins and β-Arrestin *S. K. Shenoy and R. J. Lefkowitz*
Arrestins and G proteins activate cooperative and independent signaling pathways in HEK-293 cells.

ST on the Web
Explore protein phosphorylation using PhosphoSite and Scansite in the Protein Databases section.

Separate individual or institutional subscriptions to these products may be required for full-text access.

edited by Stella Hurtley and Phil Szuromi

Incommensurate Quantum Solids

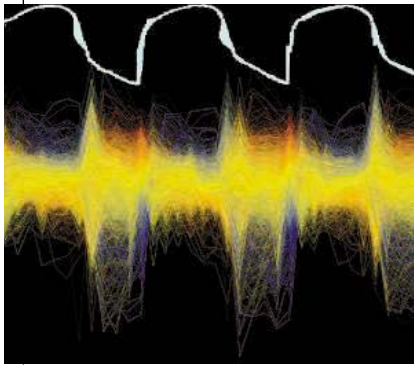
Recent experiments which showed that solid helium-4 has a nonclassical moment of inertia were interpreted in terms of the existence of a supersolid phase that can "flow" like a superfluid. **Anderson *et al.*** (p. 1164, published online 3 November) present a thermodynamic description of an "incommensurate" quantum solid, in which there is a mismatch between the number of lattice sites and the number of atoms and look at the role of interstitials and vacancies on its subsequent temperature-dependent structural and specific-heat properties. The consistency of their model with the existing experimental data prompts the authors to suggest that the ground state of solid ^{4}He may be an incommensurate quantum solid.

Graphite's Porous Relations

Numerous examples now exist of microporous materials formed from organic ligand that are linked by metal centers. **Côté *et al.*** (p. 1166) now report the formation of organic frameworks based on condensation reactions of diboronic acid that yields stacks of from graphite-like planar networks. Condensation of diboronic acid alone forms hexagonal pores 15 angstroms in diameter in which the layers are staggered, whereas condensation of this molecule with hexahydroxytriphenylene leads to larger pores (27 angstroms) with the layers stacked in an eclipsed configuration. These materials are stable to between 500° and 600°C and have surface areas of ~700 and ~1600 square meters per gram, respectively.

Cycles Underlying Growth

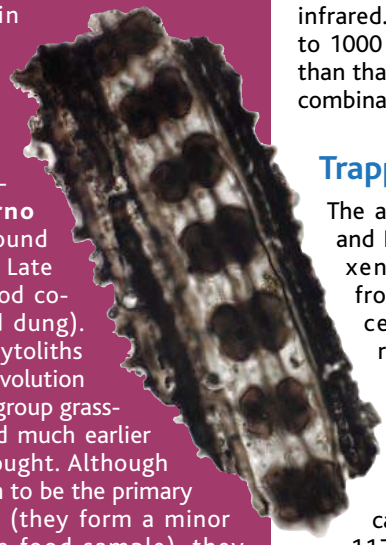
Cultures of the budding yeast *Saccharomyces cerevisiae* that are grown in limited nutrients, rather than in rich media more common in the lab, will show 4- to 5-hour cycles of respiration, as measured by O_2 consumption. **Tu *et al.*** (p. 1152, published online 27 October) found that under these conditions, more than half of the yeast genes are transcribed cyclically with a period of 300 minutes. Three large clusters of genes with related functions cycled together. The "oxidative cluster," which peaked when respiration was greatest, contains genes with roles related to protein synthesis, perhaps to make use of the high levels of adenosine triphosphate readily available at that time. The second supercluster, the "reductive/building" phase, contained many components of DNA replication and cell division, and the third "reductive/charging" group of genes contributed to nonrespiratory metabolism and protein degradation.



CREDITS (TOP TO BOTTOM): CAROLINE STRÖMBERG; TU *ET AL.*

Sauropod and Early Mammalian Grazing

The origin of grasses has been uncertain. Photosynthesis in grasses is distinct from that used in most other plants, and grasses contain specific silica structures within their cell walls (phytoliths) that can be preserved in the fossil record. **Prasad *et al.*** (p. 1177, see the Perspective by **Piperno and Sues**) have found grass phytoliths in Late Cretaceous sauropod coprolites (fossilized dung). The diversity of phytoliths is consistent with evolution of all of the crown-group grasses by this time, and much earlier than had been thought. Although grasses do not seem to be the primary food of sauropods (they form a minor component of the food sample), they might have been used by certain early mammals with enigmatic teeth.



Infrared-Radiating Carbon Nanotubes

In light-emitting diodes (LEDs), oppositely charged carriers (electrons and holes) are injected into an active region where they can recombine and release energy as photons. **Chen *et al.*** (p. 1171) show that in suspended carbon nanotubes, the local acceleration of a single type of carrier (electrons or holes) creates excitons. Under these conditions of one-dimensional confinement, excitons recombine and release radiation in the infrared. This process is 100 to 1000 times more efficient than that of electron-hole recombination in LEDs.

Trapped Below

The atmospheres of Earth and Mars have much less xenon than expected from the present concentration of other rare gases, primordial abundances, and losses and production of rare gases from outgassing and radioactive decay. **Sanloup *et al.*** (p. 1174; see the Perspective by **McMillan**) present experiments which show that large amounts of xenon can substitute for silica in quartz at high pressures and temperatures, including conditions consistent in the deeper continental crust where quartz is abundant. Xenon is released rapidly upon decompression, which makes analysis of exposed deep crustal rocks problematic.

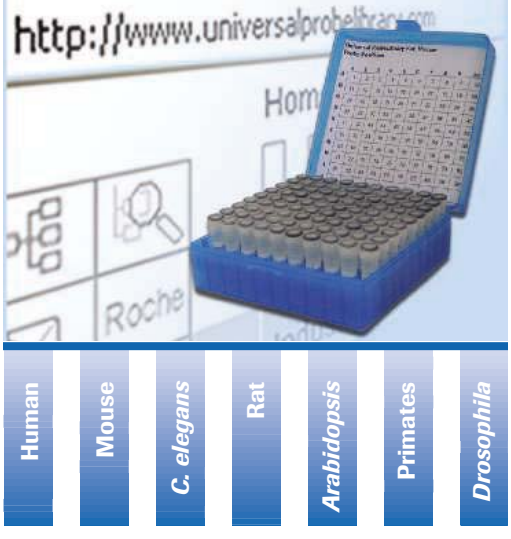
Structural View of Cytokine Interactions

Interleukin-2 (IL-2), a cytokine produced by activated T cells, promotes the proliferation, differentiation, and survival of mature T and B cells. Its actions are primarily mediated through a quaternary signaling complex that consists of IL-2, the α and β receptors (IL-2Rα, IL-2Rβ), and the γ_c chain. Now **Wang *et al.*** (p. 1159) present the extracellular structure of the quaternary complex at 2.3 angstrom resolution. Besides providing insight into IL-2 interactions that might facilitate design of IL-2 agonists and inhibitors, the structure provides a view of the γ_c receptor. This receptor is shared for IL-2, -4, -7, -9, -15, and -21 and is mutated in X-linked severe combined immunodeficiency diseases. Several mutations associated with X-SCID map to residues in the γ_c binding sites.

CONTINUED ON PAGE 1087

Universal ProbeLibrary

Simplify array validation and gene knockdown quantification



Use the online assay design center and Universal ProbeLibrary probes to generate over 2.6 million assays for multiple transcriptomes.

"All real-time PCR assays worked in the first run"

— Neven Zoric, TATAA Biocenter, Sweden

Increase lab productivity – Design custom assays online in 30 seconds and perform qPCR assays without optimization.

Obtain the benefits of probes at near-SYBR Green I prices – Use prevalidated Universal ProbeLibrary probes to detect specific amplicons – not primer-dimers or nonspecific products.

Benefit from complete assay sequence information – Obtain primer, probe, and amplicon sequences from the free, online ProbeFinder assay design software.

To learn more, and to design your next assay, visit **www.universalprobelibrary.com**

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

Circumventing Plant Pathogen Defenses

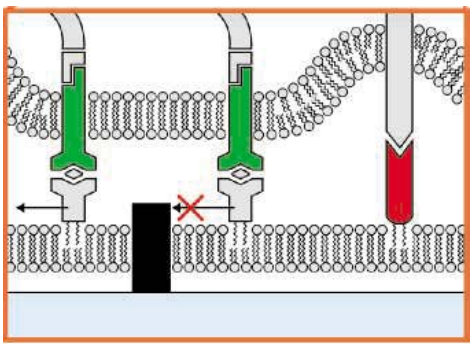
Certain plant pathogens can only initiate disease within certain plant species. What makes host species susceptible, or, conversely, what makes nonhost species resistant to infection? Studying fungi, including the one that causes potato blight, **Lipka *et al.*** (p. 1180) found a way to make *Arabidopsis*, which is normally resistant to infection, susceptible. The catalytic activity of a glycosyl hydrolase is required to keep the pathogen out, and a fail-safe program of regulated cell death further shores up defenses. The redundancy of this pathogen-defense system may help to explain its robustness.

Crucial Genes in Craniofacial Development

In humans, Williams-Beuren syndrome (WBS) results from a chromosomal deletion that usually removes 28 genes. The mutation affects craniofacial development and some aspects of cognitive and social development. Patients with WBS may be characterized by over-friendliness as well as by deficient numerical abilities. **Tassabehji *et al.*** (p. 1184, published online 3 November) have now analyzed the chromosomal disruption responsible for WBS in one patient. The results, which are supported by parallel analyses in mice, identify the gene *GTF2IRD1* in the WBS region as critical for the craniofacial defects.

A Pathway to Schizophrenia?

Schizophrenia and related mood disorders are thought to arise from a combination of genetic and environmental factors, but the identification of specific causative genes has been challenging. The *disrupted in schizophrenia 1* (*DISC1*) gene is on a short list of promising candidate-susceptibility factors, but the function of its encoded protein has been unclear. **Millar *et al.*** (p. 1187; see the Perspective by **Sawa and Snyder**) now present evidence suggesting that the DISC1 protein modulates cellular cyclic AMP (cAMP) signaling through its physical interaction with the enzyme phosphodiesterase 4B, and that disruption of this interaction may play a mechanistic role in the development of schizophrenia. Notably, cAMP signaling has previously been implicated in learning, memory, and mood in other experimental systems.



CREDIT: MOSSMAN *ET AL.*

Reshaping the Synapse

The immune synapse forms at the interface between a T cell and an antigen-presenting cell (APC) and is composed of discrete domains of stimulatory molecules and receptors critical for T cell activation. **Mossman *et al.*** (p. 1191) have imposed physical constraints on the synapse domains using a hybrid junction between a live cell and an anchored lipid bilayer (representing the APC surface). The authors directly tested the effects of membrane reorganization on the signals delivered by the synapse. Constraint of T-cell receptor ligand pairs to the periphery—rather than the center of the synapse where they normally coalesce—sustained (rather than diminished) synapse signaling, establishing a relation between the duration of T-cell receptor signals and their position in the synapse.

Golgi Inheritance in Trypanosomes

Centrins are highly conserved components of centrosomes that have long been implicated in the duplication and segregation of organelles ranging from chromosomes to mitochondria. **He *et al.*** (p. 1196, published online 27 October) have identified a new cellular structure in trypanosomes, defined by Centrin2, which is involved in the duplication of the Golgi complex. This structure has two lobes: one associated with the old Golgi, the other marking the site where the new one appears.

Not Just Jobs: ScienceCareers.org

An issue that has been with us forever, it seems, is the status of the scientific workforce. Are we training too many? Sometimes it seems that way, as well-credentialed biochemists and physicists sit marooned in postdoctoral land, waiting for the right job or any decent job. Or, as the U.S. National Science Board and the National Academies, as well as other organizations worldwide, have proclaimed, are we training too few? Or perhaps not training the "right kind" of postdoc or talking unrealistically about the prospects of those we do train? No matter where the problem lies, it's serious. A healthy scientific community is in the interest of every nation. And in the United States, the decline in competitiveness has become a matter of special concern.

In the past, *Science* and its publisher, AAAS, have approached the issue through two different institutions: one focused on readers and prospective employees and the other on employers. *Science* has addressed the gap between personal aspirations and the realities of the job market by advising young scientists about career skills and the basics of sound career management. We have helped early-career scientists get around the fixed expectations that have plagued science trainees and their mentors, in order to shape their own professional futures, no matter what employment sector they choose. For more than 10 years, *Science*'s Next Wave has been the online "knowledge environment" devoted to those objectives.

Science has also long had an online entry on the hiring side, Science Careers, which posted job opportunities from academic and industrial organizations seeking to employ scientists. In addition to its online job listings, Science Careers sponsored career fairs at which employers could meet prospective recruits, and it helped bring about decades of mostly happy scientific careers.

Having both functions was useful, but having them separate was often confusing. Five years ago, one of our authors wanted to learn how postdoctoral scholars were making use of *Science*'s Next Wave, so we rounded up half a dozen Stanford postdocs for a focus group of sorts. By the time the meeting ended, we were all wondering why AAAS had to have one place for job offerers and a separate place for job seekers. It took us awhile, but we've finally gotten around to ending this ambiguity by launching a new hybrid in the *Science*-AAAS ecosystem.

Henceforth, the functions formerly handled by *Science*'s Next Wave and Science Careers will be handled by a single Web site: ScienceCareers.org (www.sciencecareers.org). ScienceCareers.org will continue to list job opportunities and hold events that bring employers and job seekers together. On the editorial side, which is run independently out of *Science*'s News office, we will continue to help scientists and science trainees learn about the wide range of science-related careers and to provide sound science-specific advice on interviewing, preparing CVs and resumes, networking, grant writing, and all the other nonscience skills scientists need to succeed, no matter what job sector they choose. Add access to GrantsNet, our science funding database, and several other improvements and you get a product—the new ScienceCareers.org—that is, we hope, a one-stop shop for all your science career needs. And all of it will be free to anyone with an Internet connection, no matter where they live and work.

This merger is part of a sweeping overhaul of the *Science* family of Web sites (www.sciencemag.org). Our new design is intended to be easier to navigate and search as well as being visually more lively. In addition, the ScienceNOW daily news Web site will now be available to all readers without charge.

We at AAAS and *Science* believe that it benefits no one for scientists to be stuck in dead-end jobs. We merged our two career-related services to improve the fit from both ends by providing the most comprehensive science careers site on the Web. We hope that this combination will make it easier, globally, for a young scientist to find a job and for employers offering good jobs to find scientists to fill them. But our higher purpose is to help our younger readers build a career that is rewarding, fulfilling, and serves society as well as science. There is too much disappointment for comfort in that sector now, and the whole scientific community ought to be working to relieve it.

Jim Austin
Editor, ScienceCareers.org

Donald Kennedy
Editor-in-Chief, Science

10.1126/science.1122241

CREDIT: PAT N. LEWIS

Big online news from *Science*

New website – retooled and redesigned.

If you're a scientist, the online version of *Science* puts a world of essential knowledge at your fingertips. And we're now proud to announce the launch of our redesigned website, which makes it even easier to keep up with the latest breakthroughs, browse journal archives, or find career advice. New features include saved searches and content, a hotlist of the most popular article downloads, and a daily science news feed – to name just a few. Discover the new online version of *Science*. Visit **www.sciencemag.org** today.

Science
AAAS

GET THE BIG PICTURE IN *SCIENCE*

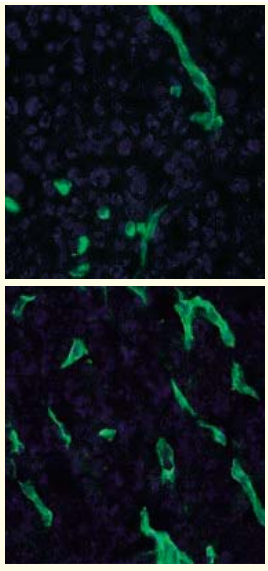
EDITORS' CHOICE

edited by Gilbert Chin

BIOMEDICINE

Resisting Arrest

An exciting class of cancer drugs acts by disrupting the growth of new blood vessels that supply solid tumors with oxygen and other essential nutrients. Because antiangiogenic therapies target genetically stable endothelial cells rather than genetically adaptable tumor cells, it had been hypothesized that tumors would be unlikely to develop resistance to these drugs. However, the results of clinical trials reveal that tumors do in fact eventually escape the growth-inhibitory effects of these drugs, although the underlying mechanisms of resistance have been unclear.



Growth of blood vessels (green) after anti-VEGFR2 (10 days, top; 4 weeks, bottom).

Casanovas *et al.* show that resistance can arise when tumors exploit a redundancy in the signaling pathways that drive angiogenesis; that is, when a drug incapacitates one pathway, tumors are able to reactivate angiogenesis through a second pathway. In a mouse model of pancreatic cancer, blocking vascular endothelial growth factor (VEGF) signaling with an antibody to VEGF receptor 2 (VEGFR2) produced a temporary arrest of tumor angiogenesis and tumor growth. Subsequently, a second wave of angiogenesis, driven by fibroblast growth factors (FGFs), led to resumption of tumor growth. Inhibiting FGF signaling during this second stage effectively blunted tumor recovery from hypoxia, and the authors propose that maximal therapeutic benefit may come from the use of drug combinations that target multiple angiogenic pathways. — PAK

Cancer Cell **8**, 299 (2005).

IMMUNOLOGY

Sharing Control

The transcription factor T-bet, encoded by *Tbx21*, is a critical regulator of T helper cell type 1 differentiation. Nevertheless, in the development of CD8 functions such as cytotoxicity and interferon-γ production, T-bet function appears to overlap with that of a related transcription factor, eomesodermin (Eomes).

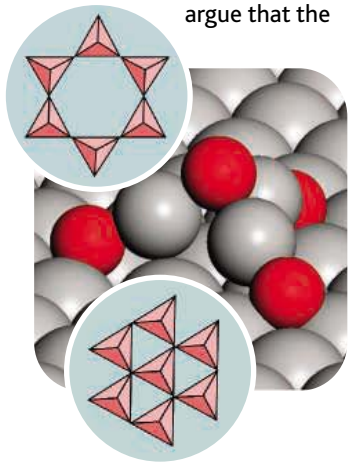
Intlekofer *et al.* explored this relationship by engineering combined genetic deficiencies of the two transcription factors. Because deletion of both *Eomes* alleles results in embryonic lethality, mice carrying heterozygous *Eomes* mutations were crossed with those carrying *Tbx21* mutations. Even with only a partial loss of Eomes, this led to significant diminution in both the number and function of memory $CD8^+$ T cells and natural killer cells, which resembles the phenotype of mice lacking the cytokine interleukin (IL)–15. Furthermore, this correlated with the loss of a marker for IL-15 responsiveness, suggesting a direct coupling of Eomes/T-bet activity with the acquisition of IL-15–directed cellular immune functions, including the long-term renewal of $CD8^+$ memory T cells. —SJS

Nat. Immunol. 10.1038/ni1268 (2005).

SURFACE SCIENCE

An Evolving Oxide Structure

One of the early triumphs of surface science was an explanation for the $p(4\times4)$ diffraction pattern observed when oxygen was adsorbed on the closest packed (111) surface of silver. In the mid-1970s, Rodiva and co-workers noted that the diagonal of the unit cell of the (111) surface of Ag_2O was within 0.3% of being four times the distance between Ag atoms on the (111) surface of the metal. With various modifications (which led to a stoichiometry of $Ag_{1.83}O$ for the overlayer), many other studies, including scanning tunneling microscopy and density functional theory (DFT) calculations, have supported a hexagonal overlayer model. Michaelidis *et al.* review the history of this problem and argue that the basic building block of the overlayer is more likely to be a pyramidal Ag_3O_4 unit. A number of nearly equivalent low-energy structures can be formed that are more stable in DFT calculations than the $Ag_{1.83}O$ model.— PDS



The Ag_3O_4 unit on the Ag substrate (main; Ag, gray; O, red) and several ways in which the pyramids may be arranged (insets).

J. Vac. Sci. Technol. A **23**, 1487 (2005).

ASTROPHYSICS

Cosmic Ringing

Gravitational attraction causes galaxies to clump together ever more strongly over time, creating a cosmic web of filaments, clusters, and superclusters. Tiny density fluctuations in the hot early universe, including ripples caused by sound waves in the plasma, have been amplified by gravity to produce the galaxy structures we see today. The faint ringing of these sound waves has been picked up in the distribution of the millions of galaxies mapped in the Sloan Digital Sky Survey.

Eisenstein *et al.* measured the correlation function of luminous red galaxies from the survey, finding a strong signal corresponding to structures with sizes of 100 Mpc, typical of superclusters of galaxies. This scale is as predicted from theories of structure in the cosmic microwave background, linking the physics of sound waves in the early universe to galaxy distributions. Eisenstein *et al.* use this correspondence to measure the overall density of matter in the universe (30%) and to infer the presence of dark energy. — JB

Astrophys. J. **633**, 560 (2005).

BIOCHEMISTRY

Pattern Recognition

Protein-protein interaction space is gradually becoming less nebulous as predictions from global two-hybrid screens of model organisms are confirmed or refuted on the basis

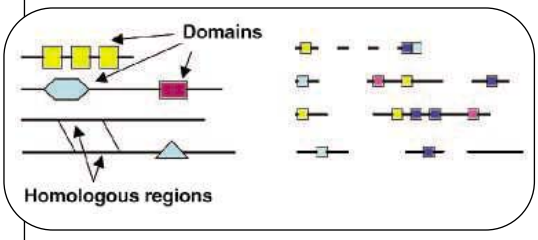
CONTINUED ON PAGE 1093

CREDITS: CASANOVAS *ET AL.*, *CANCER CELL* **8**, 299 (2005); MICHAELIDIS *ET AL.*, *J. VAC. SCI. TECHNOL. A* **23**, 1487 (2005)

of direct experimental trials or structure- and sequence-based bioinformatic analyses. On the other hand, lower-affinity interactions between smaller, peptide-sized linear motifs and their protein partners have been more difficult to catalog, in part because they are more likely to be found in the disordered (nonhelical, nonsheet) regions of protein structures and because they may be less conserved across species.

Neduva *et al.* propose a bioinformatic approach for identifying these short stretches of amino acids and apply it first to a curated set of eukaryotic linear motifs and then to the two-hybrid data set from *Drosophila*. From the sequences of a group of predicted partner proteins, they remove well-defined structural elements and homologous regions. By assessing the nonrandom appearance of peptide motifs in what remains, they obtain rankings of candidates; two of their predictions, tested in binding assays, are peptides with affinities of 20 and 40 μM, suggesting that it might now be possible to look at weak or transient interactions in a systematic fashion (see also Ruotolo *et al.*, Reports, *Science* Express, 17 November 2005). — GJC

PloS Biol. **3**, e405 (2005).



Removing domains and homologous regions (left, shapes) to uncover similar linear motifs (right, squares).

CHEMICAL ENGINEERING

A Hot Spot of Activity

The kinetics of heat flow during chemical reactions usually becomes a concern only for large-scale industrial manufacturing. However, Hyde *et al.* show that even during small-scale studies in the laboratory, local heating can lead to surprising results. Previously, the authors had found that attempts to reduce vinyl cyclohexene by palladium-catalyzed hydrogenation in supercritical carbon dioxide yielded instead a dehydrogenated product, ethyl benzene. To explore this puzzling observation, they monitored reactivity in the absence of H_2 by gas chromatography/mass spectrometry, analyzing the data using two-dimensional correlation techniques. The results suggested that an initial burst of hydrogenation generates intense heat locally, which degrades the catalyst and ignites the self-sustaining and exothermic dehydrogenation process. Thermocouple measurements confirmed that H_2 addition produces hot spots of 200°C in a catalyst column that is otherwise near room temperature. — JSY

Angew. Chem. Int. Ed. 10.1002/anie.200502049 (2005).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

Science's STKE www.stke.org

Linking Oxygen to Differentiation

Cells can detect an insufficiency of oxygen and activate signaling pathways that decrease metabolic oxidative phosphorylation or increase proliferation of blood vessels. Hypoxia also causes various stem or progenitor cells to remain in an undifferentiated state. Gustafsson *et al.* show that this latter response to hypoxia is mediated by an interaction between the oxygen-sensing mechanisms of the cell with the Notch signaling pathway. Cells use prolyl hydroxylases to sense oxygen, and these enzymes control gene expression via the transcription factor hypoxia-inducible factor-1α (HIF-1α). The membrane protein Notch can undergo proteolytic cleavage, which allows its intracellular domain (ICD) to move into the nucleus and to interact with other proteins to regulate expression. The effects of hypoxia on differentiation of cultured mouse muscle precursor cells or primary rat neural stem cells depend on Notch signaling and were prevented by an inhibitor of the protease that generates the Notch ICD. The authors propose that HIF-1α may interact with the Notch ICD and showed that they do so in vitro. Furthermore, chromatin immunoprecipitation analyses showed that HIF-1α is recruited to the promoter regions of Notch-responsive genes in cells exposed to hypoxia, provided that Notch signaling was also activated. These results help explain the mechanisms that couple oxygen sensing to control of differentiation.— LBR

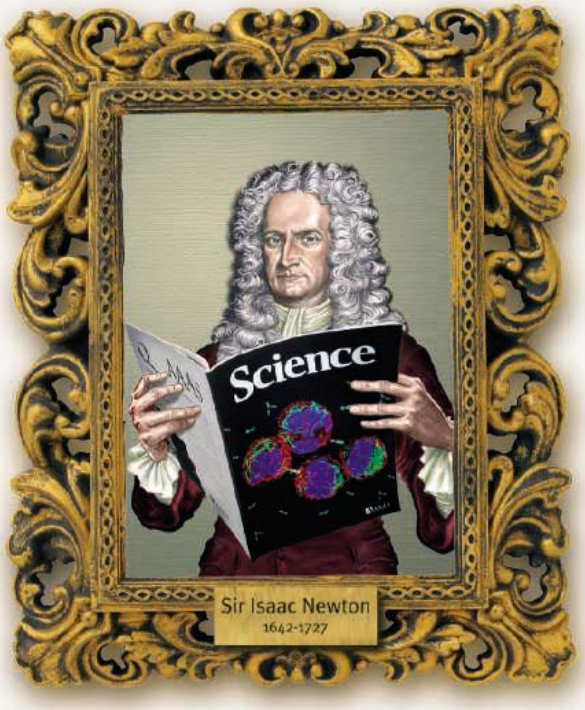
Dev. Cell **9**, 617 (2005).

CREDITS: NEDUVA *ET AL.*, *PLOS BIOL.* 3, E405 (2005)

ScienceCareers.org
now with Next Wave

ScienceCareers.org is the leading careers resource for scientists. And now it offers even more. In addition to a brand new website with easier navigation, ScienceCareers.org now includes Next Wave, the essential online careers magazine. Next Wave is packed with features and articles to help advance your science career – all for free.

- Hundreds of job postings
- Career tools from Next Wave
- Grant information
- Resume/CV Database
- Career Forum



www.sciencemag.org

Science

1200 New York Avenue, NW
Washington, DC 20005
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6500, FAX 202-371-9227

Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

Subscription Services For change of address, missing issues, new orders and renewals, and payment questions: 800-731-4939 or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 1811, Danbury, CT 06813 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

Institutional Site Licences please call 202-326-6755 for any questions or information

Reprints: Author Inquiries 800-635-7181
Commercial Inquiries 803-359-4578
Corrections 202-326-6501

Permissions 202-326-7074, FAX 202-682-0816

Member Benefits Bookstore: AAAS/BarnesandNoble.com bookstore www.aaas.org/bn; Car purchase discount: Subaru VIP Program 202-326-6417; Credit Card: MBNA 800-847-7378; Car Rentals: Hertz 800-654-2200 CDP#343457, Dollar 800-800-4000 #AA1115; AAAS Travels: Betchart Expeditions 800-252-4910; Life Insurance: Seabury & Smith 800-424-9883; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org (for general editorial queries)
science_letters@aaas.org (for queries about letters)
science_reviews@aaas.org (for returning manuscript reviews)
science_bookrevs@aaas.org (for book review queries)

Published by the **American Association for the Advancement of Science (AAAS)**, *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by the AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science and innovation throughout the world for the benefit of all people. The goals of the association are to: foster communication among scientists, engineers and the public; enhance international cooperation in science and its applications; promote the responsible conduct and use of science and technology; foster education in science and technology for everyone; enhance the science and technology workforce and infrastructure; increase public understanding and appreciation of science and technology; and strengthen support for the science and technology enterprise.

Information for Contributors

See pages 135 and 136 of the 7 January 2005 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

EDITOR-IN-CHIEF **Donald Kennedy**
EXECUTIVE EDITOR **Monica M. Bradford**
DEPUTY EDITORS **R. Brooks Hanson, Katrina L. Kelner**
NEWS EDITOR **Colin Norman**

Editorial SUPERVISORY SENIOR EDITORS Barbara Jasny, Phillip D. Szuromi; SENIOR EDITOR/PERSPECTIVES Lisa D. Chong; SENIOR EDITORS Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Beverly A. Purnell, L. Bryan Ray, Guy Riddihough (Manila), H. Jesse Smith, Valda Vinson, David Voss; ASSOCIATE EDITORS Marc S. Lavine (Toronto), Jake S. Yeston; ONLINE EDITOR Stewart Wills; CONTRIBUTING EDITOR Ivan Amato; ASSOCIATE ONLINE EDITOR Tara S. Marathe; BOOK REVIEW EDITOR Sherman J. Suter; ASSOCIATE LETTERS EDITOR Etta Kavanagh; INFORMATION SPECIALIST Janet Kegg; EDITORIAL MANAGER Cara Tate; SENIOR COPY EDITORS Jeffrey E. Cook, Harry Jach, Barbara P. Ordway; COPY EDITORS Cynthia Howe, Alexis Wynne Mogul, Jennifer Sills, Trista Wagoner; EDITORIAL COORDINATORS Carolyn Kyle, Beverly Shields; PUBLICATION ASSISTANTS Ramatoulaye Diop, Chris Filiatreau, Joi S. Granger, Jeffrey Hearn, Lisa Johnson, Scott Miller, Jerry Richardson, Brian White, Anita Wynn; EDITORIAL ASSISTANTS E. Annie Hall, Lauren Kmec, Patricia M. Moore, Brendan Nardozzi, Michael Rodewald; EXECUTIVE ASSISTANT Sylvia S. Kihara; ADMINISTRATIVE SUPPORT Patricia F. Fisher

News SENIOR CORRESPONDENT Jean Marx; DEPUTY NEWS EDITORS Robert Coontz, Jeffrey Mervis, Leslie Roberts, John Travis; CONTRIBUTING EDITORS Elizabeth Culotta, Polly Shulman; NEWS WRITERS Yudhijit Bhattacharjee, Adrian Cho, Jennifer Couzin, David Grimm, Constance Holden, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Andrew Lawler (New England), Greg Miller, Elizabeth Pennisi, Robert F. Service (Pacific NW), Erik Stokstad; Carolyn Gramling (intern); CONTRIBUTING CORRESPONDENTS Marcia Barinaga (Berkeley, CA), Barry A. Cipra, Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Robert Irion, Mitch Leslie (NetWatch), Charles C. Mann, Evelyn Strauss, Gary Taubes, Ingrid Wickelgren; COPY EDITORS Linda B. Felaco, Rachel Curran, Sean Richardson; ADMINISTRATIVE SUPPORT Scherraine Mack, Fannie Groom BUREAUS: Berkeley, CA: 510-652-0302, FAX 510-652-1867, New England: 207-549-7755, San Diego, CA: 760-942-3252, FAX 760-942-4979, Pacific Northwest: 503-963-1940

Production DIRECTOR James Landry; SENIOR MANAGER Wendy K. Shank; ASSISTANT MANAGER Rebecca Doshi; SENIOR SPECIALISTS Jay R. Covert, Chris Redwood **Preflight** DIRECTOR David M. Tompkins; MANAGER Marcus Spiegler; SPECIALIST Jessie Mudjitaba

Art DIRECTOR Joshua Moglia; ASSOCIATE ART DIRECTOR Kelly Buckheit; ILLUSTRATORS Chris Bickel, Katharine Sutliff; SENIOR ART ASSOCIATES Holly Bishop, Laura Creveling, Preston Huey; ASSOCIATE Nayomi Kevitiyagala; PHOTO RESEARCHER Leslie Blizard

***Science* International**

Europe (science@science-int.co.uk) EDITORIAL: INTERNATIONAL MANAGING EDITOR Andrew M. Sugden; SENIOR EDITOR/PERSPECTIVES Julia Fahrenkamp-Uppenbrink; SENIOR EDITORS Caroline Ash (Geneva: +41 (0) 222 346 3106), Stella M. Hurtley, Ian S. Osborne, Stephen J. Simpson, Peter Stern; ASSOCIATE EDITOR Joanne Baker EDITORIAL SUPPORT Alice Whaley; Deborah Dennison ADMINISTRATIVE SUPPORT Janet Clements, Phil Marlow, Jill White; NEWS: INTERNATIONAL NEWS EDITOR Eliot Marshall DEPUTY NEWS EDITOR Daniel Clery; CORRESPONDENT Gretchen Vogel (Berlin: +49 (0) 30 2809 3902, FAX +49 (0) 30 2809 8365); CONTRIBUTING CORRESPONDENTS Michael Balter (Paris), Martin Enserink (Amsterdam and Paris); INTERN Michael Schirber

Asia Japan Office: Asca Corporation, Eiko Ishioka, Fusako Tamura, 1-8-13, Hirano-cho, Chuo-ku, Osaka-shi, Osaka, 541-0046 Japan; +81 (0) 6 6202 6272, FAX +81 (0) 6 6202 6271; asca@os.gulf.or.jp JAPAN NEWS BUREAU: Dennis Normile (contributing correspondent, +81 (0) 3 3391 0630, FAX 81 (0) 3 5936 3531; dnormile@gol.com); CHINA REPRESENTATIVE Hao Xin, + 86 (0) 10 6307 4439 or 6307 3676, FAX +86 (0) 10 6307 4358; haoxin@earthlink.net; SOUTH ASIA Pallava Bagla (contributing correspondent +91 (0) 11 2271 2896; pbagla@vsnl.com); ASIA Richard Stone +66 2 662 5818 (rstone@aaas.org)

EXECUTIVE PUBLISHER **Alan I. Leshner**
PUBLISHER **Beth Rosner**

Fulfillment & Membership Services (membership@aaas.org) DIRECTOR Marlene Zendell; MANAGER Waylon Butler; SYSTEMS SPECIALIST Andrew Vargo; SPECIALISTS Pat Butler, Laurie Baker, Tamara Alfson, Karena Smith, Vicki Linton; CIRCULATION ASSOCIATE Christopher Refice

Business Operations and Administration DIRECTOR Deborah Rivera-Wienhold; BUSINESS MANAGER Randy Yi; SENIOR BUSINESS ANALYST Lisa Donovan; BUSINESS ANALYST Jessica Tierney; FINANCIAL ANALYST Michael LoBue, Farida Yeasmin; RIGHTS AND PERMISSIONS: ADMINISTRATOR Emilie David; ASSOCIATE Elizabeth Sandler; MARKETING: DIRECTOR John Meyers; MARKETING MANAGERS Darryl Walter, Allison Pritchard; MARKETING ASSOCIATES Julianne Wielga, Mary Ellen Crowley, Catherine Featherston; DIRECTOR OF INTERNATIONAL MARKETING AND RECRUITMENT ADVERTISING Deborah Harris; INTERNATIONAL MARKETING MANAGER Wendy Sturley; MARKETING/MEMBER SERVICES EXECUTIVE: Linda Rusk; JAPAN SALES Jason Hannaford; SITE LICENSE SALES: DIRECTOR Tom Ryan; SALES AND CUSTOMER SERVICE Mehan Dossani, Kiki Forsythe, Catherine Holland, Wendy Wise; ELECTRONIC MEDIA: MANAGER Lizabeth Harman; PRODUCTION ASSOCIATES Sheila Mackall, Amanda K. Skelton, Lisa Stanford, Nichele Johnston; APPLICATIONS DEVELOPER Carl Saffell

Advertising DIRECTOR WORLDWIDE AD SALES Bill Moran

Product (science_advertising@aaas.org); MIDWEST Rick Bongiovanni: 330-405-7080, FAX 330-405-7081 • WEST COAST/W. CANADA B. Neil Boylan (Associate Director): 650-964-2266, FAX 650-964-2267 • EAST COAST/E. CANADA Christopher Breslin: 443-512-0330, FAX 443-512-0331 • UK/EUROPE/ASIA Tracey Peers (Associate Director): +44 (0) 1782 752530, FAX +44 (0) 1782 752531 JAPAN Mashy Yoshikawa: +81 (0) 33235 5961, FAX +81 (0) 33235 5852 ISRAEL Jessica Nachlas +9723 5449123 • TRAFFIC MANAGER Carol Maddox; SALES COORDINATOR Deiandra Simms

Classified (advertise@sciencecareers.org); U.S.: SALES DIRECTOR Gabrielle Boguslawski: 718-491-1607, FAX 202-289-6742; INSIDE SALES MANAGER Daryl Anderson: 202-326-6543; WEST COAST/MIDWEST Kristine von Zedlitz: 415-956-2531; EAST COAST Jill Downing: 631-580-2445; CANADA, MEETINGS AND ANNOUNCEMENTS Kathleen Clark: 510-271-8349; LINE AD SALES Emnet Tesfaye: 202-326-6740; SALES COORDINATORS Erika Bryant; Rohan Edmonson Christopher Normile, Joyce Scott, Shirley Young; INTERNATIONAL: SALES MANAGER Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532; SALES Christina Harrison, Svitlana Barnes; SALES ASSISTANT Helen Moroney; JAPAN: Jason Hannaford: +81 (0) 52 789 1860, FAX +81 (0) 52 789 1861; PRODUCTION: MANAGER Jennifer Rankin; ASSISTANT MANAGER Deborah Tompkins; ASSOCIATES Christine Hall; Amy Hardcastle; PUBLICATIONS ASSISTANTS Robert Buck; Natasha Pinol

AAAS Board of Directors RETIRING PRESIDENT, CHAIR Shirley Ann Jackson; PRESIDENT Gilbert S. Omenn; PRESIDENT-ELECT John P. Holdren; TREASURER David E. Shaw; CHIEF EXECUTIVE OFFICER Alan I. Leshner; BOARD Rosina M. Bierbaum; John E. Burris; John E. Dowling; Lynn W. Enquist; Susan M. Fitzpatrick; Richard A. Meserve; Norine E. Noonan; Peter J. Stang; Kathryn D. Sullivan

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

New!
Applied Biosystems 9800 Fast PCR System.

PCR in just 25 minutes.

The 9800 Fast PCR System is the first fully integrated solution delivering fast PCR performance in a standard 96-well format. The system reduces PCR reaction time from two hours to 25 minutes or less—advancing you quickly to the next step of your research. With the fast-optimized system including the 9800 thermal cycler, GeneAmp® reagents, integrated consumables, and world-class technical s upport, you can count on fast, reliable results. Put your trust in the 9800 Fast PCR System for faster PCR performance—visit http://info.appliedbiosystems.com/**9800**

Applied Biosystems

Looking for a meeting with world-renowned research in science, technology, and engineering?

Then head to St. Louis for the AAAS Annual Meeting, 16–20 February 2006! It's the premier forum for world-renowned scientific discovery and discussion.

Photos: Courtesy: (Left to Right) 1) NSF; ©GTEC; 2) NIST ©Geoffrey Wheeler; 3) © Brian Prechtel, ARS/USDA; 4) NSF; ©John Crespi, Pennsylvania State Physics, Licensed as Creative Commons Attribution-Share-Alike 2.5; 5) NSF ©Photo by Gary Meek.

No other scientific meeting provides the scope of science, engineering, and technology from the world's leading researchers that is found at the AAAS Annual Meeting. At this truly interdisciplinary event you can unravel the mysteries of how insects fly, explore the interplay between real and virtual worlds, and learn about research into ways to prevent severe storm damage. Keynote speakers from around the world will take you to new frontiers … and beyond.

Highlights include:

Nearly 200 symposia in many critical areas, from sustainability and reduction in poverty around the world, to national security, health, energy, agriculture, space, human behavior, evolution, and education.

Plenary & Topical Lecturers such as:

- **Nobel Prize winner Peter Agre**, Professor of Biological Chemistry, Johns Hopkins University School of Medicine
- **Jeffrey D. Sachs**, Director, Earth Institute, Columbia University and Director, The UN Millennium Project
- **Sir Partha Dasgupta**, Professor of Economics, University of Cambridge

Special Events

- **Beyond Pi: Grand Challenges in the Mathematical Sciences** highlights the challenges faced by mathematicians from both within and outside their field.
- **Physics and Economics of Virtual Worlds** examines the video game industry, a major force in the entertainment sector.
- **2006 Nanotechnology Seminar** examines promising applications, potential health risks, and broad societal implications.
- **Forum for School Science** focuses on student assessment as a driver of change.

Visit our Web site for full details:
www.aaasmeeting.org

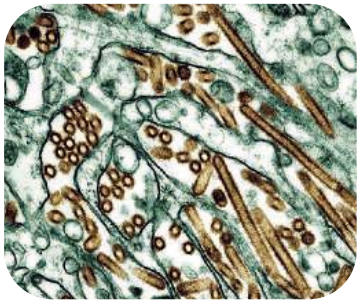
AAAS
ADVANCING SCIENCE, SERVING SOCIETY

NetWatch

edited by Mitch Leslie

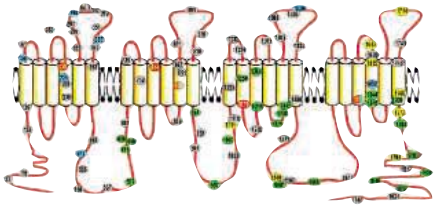
NET RESOURCES

Disease Daily



The bird flu virus (H5N1) spreading from Asia to Europe has the world worried about a possible human flu pandemic. For the latest on avian influenza and other microbial threats, click over to the Web site of the Center for Infectious Disease Research and Policy at the University of Minnesota, Minneapolis. The clearinghouse holds information on more than a dozen illnesses, from SARS to potential bioterrorism weapons such as bubonic plague. Visitors can read daily news reports, abstracts of recent papers, and other documents. For some diseases, you'll find backgrounders that describe symptoms, epidemiology, diagnosis, and treatment. Above, the gold cylinders in this photo are H5N1 viruses.

www.cidrap.umn.edu



DATABASE

Out of Synch

People with long QT syndrome can faint or die suddenly because their ventricles are prone to rapid contractions. The disease results from mutations that make heart muscle cells repolarize tardily after firing. Find out more about the DNA defects behind long QT syndrome and eight other arrhythmias at Gene Connection for the Heart, hosted by the IRCCS Fondazione Salvatore Maugeri in Pavia, Italy. The database describes the diseases and profiles each mutation, indicating its location and how it changes the gene and the protein. The diagram above shows where mutations alter the SCN5A protein, which allows sodium ions into heart cells during contraction.

pc4.fsm.it:81/cardmoc

TOOLS

Stacking Gene Chips

Microarrays reveal which genes crank up or slow down in diseases such as diabetes and cancer, but they yield a torrent of data that leaves many researchers feeling swamped. A new site called L2L (for "list-to-list") from the University of Washington, Seattle, can help scientists cope with the flood. Users plug in their lists of regulated genes, and L2L compares them to more than 350 other lists compiled from published microarray papers. The output highlights common patterns of gene expression that suggest underlying molecular mechanisms. L2L can help researchers tease apart the effects of complex diseases on gene activity.

depts.washington.edu/l2l

WEB LOGS

Talking Physics

What are people saying about the latest papers on the physics preprint site arXiv or the report that the solar system's putative 10th planet has a moon? Keep up with the latest physics chatter at this pair of Web logs. For nearly 3 years, mathematical physicist John Baez of the University of California, Riverside, has discoursed on books and papers that catch his interest,* particularly if they relate to gravitational theory. Recent indications that a moon orbits what might be the 10th planet—it hasn't received official recognition yet—inspired him to write a tutorial on the solar system's suburbs, complete with diagrams and photos.

More provocative is Not Even Wrong,† in which mathematician Peter Woit of Columbia University takes a critical look at string theory and other timely topics in physics and science. In a recent commentary, he compares string theory to "intelligent design," arguing that we might have passed the point at which further work to understand the theory "stops being science and it too starts being a nonscientific activity pursued for sociological and psychological reasons."

* math.ucr.edu/home/baez

† www.math.columbia.edu/~woit/wordpress

RESOURCES

Death in the Woods

The killer stalking the cool, damp forests of the U.S. West Coast sounds familiar: a rootless drifter that slays silently and often gets around by hitchhiking. The wrongdoer is the funguslike parasite *Phytophthora ramorum*, which causes sudden oak death and has attacked oaks and other woodland plants in 14 counties in California and one in southern Oregon. This site from the California Oak Mortality Task Force features a chronology and maps that track *P. ramorum;* the organism first appeared in 1995, and its origins are unknown. Visitors can also learn how to diagnose infestations and read about the pest's impact on U.S. nurseries. A gallery of species felled by the pathogen includes this aerial photo (right) of dying and dead tanoaks in California's Los Padres National Forest near Monterey.

www.suddenoakdeath.org

Send site suggestions to netwatch@aaas.org. Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): C. GOLDSMITH; IRCCS FONDAZIONE SALVATORE MAUGERI; SUSAN FRANKEL

This Week

PAGE 1104
Dalai Lama at neuroscience meeting

1105
Win one, lose one on the ID front

STEM CELLS

Collaborators Split Over Ethics Allegations

Allegations of ethical lapses have broken up a high-profile collaboration in human cloning and embryonic stem (ES) cell research and have put others on hold. Gerald Schatten, a stem cell researcher at Pittsburgh University School of Medicine in Pennsylvania, announced on 12 November that he would no longer work with Woo-Suk Hwang, leader of the Seoul National University team that was the first to report deriving human ES cells from cloned embryos (*Science*, 12 March 2004, p. 1669). Schatten had collaborated with Hwang since early 2004, and he was listed as a senior author on a second *Science* paper, published online 19 May 2005, that reported the first derivations of human ES cells carrying the genome of patients suffering from disease. He was also slated to play a leading role in the newly formed World Stem Cell Hub that the two researchers announced in October (*Science*, 21 October, p. 419). Schatten's statement came just days after another Hwang collaborator was investigated in connection with illegal payments to egg donors.

Schatten accuses Hwang of misleading him about the source of oocytes for the 2004 *Science* paper. (The team inserted a nucleus from a skin or other cell into an oocyte from which the DNA had been removed.) Schatten, who was not an author of the 2004 paper, did not detail his charges, but questions had been raised earlier about the source of the oocytes. In the first *Science* paper, the researchers said that their single cell line was the result of 242 tries with oocytes donated by 16 women. Shortly after the paper was published, *Nature* reported allegations that two junior members of the lab had donated oocytes for the work. Such a donation, although not illegal, would raise ethical flags because lab members might feel pressure from senior members or might think they could benefit, for example by being named co-author. Hwang and others involved in the research denied the allegations, saying that no lab members had donated oocytes to the project and that none of the donors had been paid.

Schatten said in a 12 November statement that he had believed Hwang's explanation, but he now has doubts. "Regrettably, yesterday information came to my attention suggesting that misrepresentations might have occurred relating to those oocyte donations," he said.

The flap apparently grew out of a criminal investigation involving Hwang's collaborator Sung-Il Roh, a fertility specialist at MizMedi Hospital in Seoul, who helped collect many of the oocytes Hwang's team used in the 2005 *Science* paper. On 8 November, South Korean media reported that police were investigating whether Roh was involved in illegal payments for oocytes that were fertilized and implanted into infertile women. South Korea's new bioethics law, which went into effect in January, prohibits any payment for donated oocytes. On 10 November, Schatten wrote to editors at *Science* assuring them that no donors had been paid for eggs used in either paper. Two days later, he announced that he was ending the collaboration because of a "breach of trust."

Happier times. Gerald Schatten (*right*) has ended his collaboration with Woo-Suk Hwang, accusing Hwang of misleading him about oocyte donors.

Donald Kennedy, editor-in-chief of *Science*, issued a statement saying that the journal is "taking the allegations very seriously." Editors "exercised unusually careful diligence" before accepting both papers from Hwang's group, he says, and will take appropriate action if the allegations are substantiated.

In an e-mail, Hwang declined to comment on Schatten's allegations other than to say he is investigating the matter and will announce his conclusions as soon as possible. Moon-il Park, chair of the institutional review board at Hanyang University Hospital, where the donor eggs were collected for the 2004 paper, confirmed in an e-mail that he stands by previous statements to *Science*, saying that no one from Hwang's team was among the 16 donors (*Science*, 14 May 2004, p. 945). The bioethics law was not in effect then, so any payment, although ethically questionable, would have been legal.

In his statement, Schatten says he also found mistakes in a table from the paper published in May but that the mistake does not change the paper's conclusions.

Hans Schöler of the Max Planck Institute for Molecular Medicine in Münster, Germany, who has visited Hwang's lab and had been discussing a possible collaboration, says his interactions with Hwang have given him no reason to doubt Hwang's honesty. But he adds, "If the accusations turn out to be correct, … they will affect the whole field." For example, Schöler says, any whiff of impropriety will damage ongoing efforts to convince German officials that scientists should be allowed to collaborate with Hwang. "One argument will be that if Hwang was dishonest with a collaborator, how dishonest will he be toward the public?" he says.

Insoo Hyun, a bioethicist at Case Western Reserve University in Cleveland, Ohio, says Schatten's allegations shocked him. Hyun spent several months with the Seoul group this summer studying the ethical standards they currently use. Although he did not look specifically into the collection of oocytes for the 2004 paper, he says he was impressed that the group's current guidelines go beyond those of many U.S. institutions. He has also advised Hwang on bioethics issues surrounding the World Stem Cell Hub project. He says his colleagues in South Korea are dismayed as well and are trying to find out the details of Schatten's concerns.

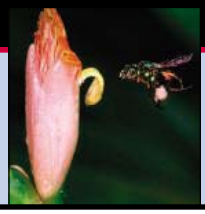
—GRETCHEN VOGEL

With reporting by Dennis Normile in Tokyo.

CREDIT: AHN YOUNG-JOON/AP PHOTO

1106
When will oil production peak?

1109
Pollinators and evolution



1112
How serious is the H5N1 threat?

U.S. HIGHER EDUCATION

Professor Sues University Over Building He Is Funding

A cancer drug that chemist Robert Holton invented has reaped more than $350 million in royalties. But his efforts to transform part of that windfall into an expanded chemistry program and new building for his school, Florida State University (FSU) in Tallahassee, has led to a lawsuit and a pitched battle between the school's chemistry department and its administration.

The suit, Holton says, was a last resort after the university backed out of a 2002 agreement to construct a five-story building to study his kind of chemistry—molecular interactions and the synthesis of new molecules—as well as to double the size of the synthetic chemistry faculty. Instead, the university plans to use the money to construct and equip a general chemistry building different from Holton's vision. "Onerous" demands by Holton forced the administration to "adopt a new direction," wrote FSU President T. K. Wetherell in a July letter to trustees. Wetherell took the helm after planning for the building was already well under way.

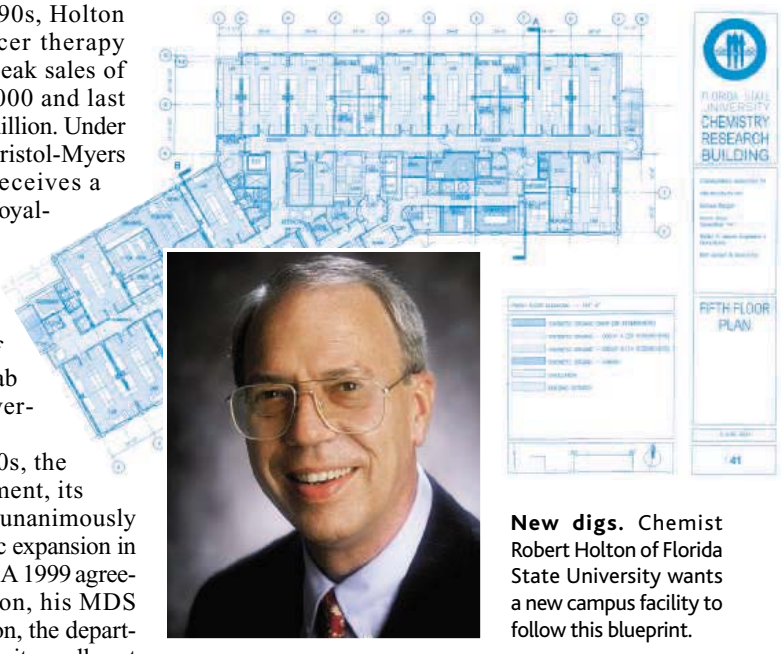
"I am disappointed and embarrassed," says Holton, who offered $18.5 million from his lab account, which the university says it won't return, for the $67 million facility. "We thought we had it worked out." About Wetherell, a former politician and lobbyist with whom Holton has clashed, "I'm better off saying nothing."

In the early 1990s, Holton invented the cancer therapy Taxol, which had peak sales of $1.6 billion in 2000 and last year totaled $256 million. Under agreements with Bristol-Myers Squibb, Holton receives a 40% share of the royalties and the FSU chemistry and biochemistry department a 30% share, of which half flows to Holton's lab account. The university gets 30%.

In the late 1990s, the chemistry department, its account swelling, unanimously agreed to a dramatic expansion in synthetic chemistry. A 1999 agreement among Holton, his MDS Research Foundation, the department, and the university spells out how the money would be spent. Modified in 2002, the pact included 165 fume hoods for toxic chemicals, at a cost of up to $50,000 apiece. The state has chipped in $11 million but is not a party to the suit.

University administrators say Holton has micromanaged the plans, including making "parking demands." Holton denies that he's made further requests and says, "we have not added a single thing" to the 2002 agreement.

Regardless, says FSU general counsel Betty Steffens, "there is nothing to return" when it comes to Holton's lab account funds because that money belongs to the university. FSU has agreed to return $5 million of the $11 million donated by the foundation in ▶



New digs. Chemist Robert Holton of Florida State University wants a new campus facility to follow this blueprint.

CREDITS: O'BRIEN/ATKINS ASSOCIATES PA, PROVIDED BY FSU; (INSET) COURTESY OF ROBERT HOLTON

POLITICS

Antiterror Law Intrusive, U.K. Academic Groups Warn

CAMBRIDGE, U.K.—Several scientific and academic groups objected last week to a tough antiterrorism law making its way through the U.K. Parliament. The critics argue that academic freedom could be endangered by language stating that if lecturers and lab chiefs "know or suspect" that their students are terrorists, they must withhold from them knowledge of "noxious substances."

In a statement on 12 November, the Royal Society of Chemistry (RSC) warned that, "as drafted, the bill could make it illegal to teach about the safe use and handling of chemicals with explosive properties." The RSC wants to see some sections "redrafted." Neville Reed, director of RSC community and members' services, says, "We understand the reasoning behind the bill, … but because it is written so broadly, there's a danger of encompassing things that are part of normal teaching." A lecturer might be put in the position of having to demand why a question is being asked, rather than saying, "That's an interesting question."

The Association of University Teachers (AUT) also lobbied for changes in the bill's language, arguing that there is a "huge risk that entirely legitimate forms of academic enquiry will be criminalized." AUT head of parliamentary affairs John Whitehead cited three clauses that aroused concern, one of which has now been rewritten to narrow a prohibition against the "glorification" of terrorism so that it applies only to people who clearly intend to engage in terrorism. But he says the clause that refers to people whom an instructor "knows or suspects" of having bad intentions needs to be changed simply to "knows."

The bill, introduced last month by the government of Prime Minister Tony Blair, was passed by the House of Commons last week, but only after critics forced through an amendment cutting back the amount of time a terrorism suspect may be held without charge from 90 to 28 days. Now it goes to the House of Lords, where observers say further revisions are likely.

—ELIOT MARSHALL

two separate donations years apart. The rest, says Steffens, was not directed toward a building dedicated to synthetic chemistry. Michael Devine, the foundation's executive director, disagrees, saying its two donations were earmarked for a program focused on synthetic chemistry.

The fight between Holton and Wetherell has spilled over onto the university's 36-person chemistry department. On 31 August, the department passed a resolution in which it "vehemently objected" to plans for a broader chemistry building and requested that its faculty "be the driving force in determining the best use of its endowed monies."

The administration's reaction was swift and harsh, according to three faculty members. One, speaking anonymously, said that Joseph Travis, FSU's dean of the College of Arts and Sciences, suggested to the department that "if the resolution was delivered [to Wetherell], the [department] chair would be removed, the department put in receivership, and funding would be frozen." "There's definitely an intimidation factor," says Marie Krafft, an FSU synthetic chemist and Holton's wife.

Travis says he was "not telling them what to do." Although he declined to offer specifics, he explained that "my goal was to urge them to think through the consequences of saying a building was unacceptable."

With just four synthetic chemists on its faculty, FSU is not considered a top-20 player in the field, says Steve Burke, a synthetic chemist at the University of Wisconsin, Madison. Burke believes improved facilities and additional faculty could make a difference, however, and the 2002 plans included $20 million for four new synthetic chemistry professorships. Steffens now says FSU does not plan to fill those posts.

Holton and the MDS Research Foundation would prefer to see the original building constructed. "But if they're not going to do that," says Devine, "we want the money back." The suit was filed 8 November in the 2nd Circuit Court for Leon County, Florida.

–JENNIFER COUZIN

AFRICAN SCIENCE

A Move to Revamp Elite Institutions Across the Continent

NAIROBI, KENYA—Although Africa faces daunting challenges, the continent's science academies are rarely asked to advise governments on major issues—and they seldom volunteer to do so. Instead, these elite bodies have tended to focus mainly on the concerns of their aging memberships, moving along in what one scientist calls "geological time." But a new effort, aided by outside money and expertise, is setting out to revitalize African academies and give science more influence. The organizers would like these institutions to provide evidence-based advice to African leaders on complex issues, for example, on how to respond to pockets of resistance to polio vaccination, doubts about antiretroviral treatments for HIV/AIDS, and confusion about genetically modified crops.

"Africa's science academies can no longer afford to be private clubs for aging men," says mathematician Mohamed Hassan of Sudan, president of the African Academy of Sciences and executive director of the Academy of Sciences for the Developing World. "They should address important issues, reach out to women and younger scientists, and learn how to communicate."

This agenda fits in with the goals of the African Science Academy Development Initiative, which brought about 150 African academicians, government officials, and outside experts to an unprecedented meeting here on 7 to 9 November. It was the public kickoff of a 10-year effort—coordinated by the U.S. National Academies (NAS) with the support of a $20 million grant from the Bill and Melinda Gates Foundation—to make the continent's 13 academies more active and relevant.

Three academies—in South Africa, Nigeria, and Uganda—have been the first to take part in the NAS program at an intensive level, receiving financial support as well as training and partnering programs for staff.

Synergy. Presidents of the Nigerian and South African academies, Gabriel Ogunmola (*left*) and Robin Crewe, explored common ground in Nairobi.

Nigeria's academy has expanded its staff to 10, updated its information system, and strengthened its influence on national science policy, said its president, chemist Gabriel B. Ogunmola.

In South Africa, the NAS support that began last spring has enabled that nation's academy to grow, says medical biochemist Wieland Gevers, a former president who recently became the academy's executive officer. The academy, which has 49 women among its 235 members, has hired four new staffers, is improving its Web site, linking to other African academies, and gearing up to produce its first reports on topical issues. South African academy president Robin Crewe, an entomologist, says plans call for an assessment of the nation's scientific journals and a study of the impact of nutrition on health—a sensitive issue because some officials have emphasized nutritional supplements more than antiretroviral drugs for treating HIV/AIDS. And it's hard to imagine a broader mandate than one request: The government wants to know how science could help alleviate poverty.

Uganda, smaller than the other initial NAS partners, has used the collaboration to transform its once-tiny academy, says President Paul Mugambi. He says the government has embraced the concept of making the academy "a major source of policy advice" on scientific issues. Several other academies expect to receive NAS seed money for strategic planning, including those in Cameroon, Senegal, Ghana, and Kenya, as well as the African Academy of Sciences in Nairobi.

The NAS initiative dovetails with separate efforts to improve African universities as well as a wider plan to improve and better coordinate research in Africa. Under the African Union's New Partnership for Africa's Development, science ministers this fall endorsed a scheme to increase R&D budgets and establish centers of excellence. Senegal's research minister, Yaye Kene Gassama Dia, a professor of plant biotechnology at the University of Dakar who helped develop the plan, told academicians that Africa's science ministers want to strengthen the link between researchers and policy development. "The academies must build on this commitment," she said.

The academicians plan to meet next fall in Cameroon to assess their progress.

–ROBERT KOENIG

Robert Koenig is a science writer in South Africa.

CREDIT: U.S. NATIONAL ACADEMIES

INFLUENZA

Meeting Seeks Global Consensus, Highlights Global Disparities

GENEVA, SWITZERLAND—If anyone needed more evidence that the threat of a flu pandemic has become a global priority, last week's meeting at World Health Organization (WHO) headquarters here provided it. Diplomats and health experts from more than 110 countries and a dozen international organizations expressed their worries, the World Bank and other organizations drew out their checkbooks, and more than 100 journalists queued to interview key speakers. The words "unprecedented" and "historic" were on many participants' lips.

All together now. Flu worries rose to the top of the international agenda at a meeting at WHO headquarters last week.

Although many pleaded for global solidarity, some glaring disparities remain. In particular, the meeting highlighted the rift between rich and poor countries' abilities to battle a pandemic. Western nations are stockpiling antiviral drugs and developing vaccines, leaving poor and middle-income countries to worry that they won't have access to these potential lifesavers.

The meeting, co-organized by WHO, the U.N. Food and Agriculture Organization, the World Organization for Animal Health, and the World Bank, aimed to stimulate countries to draw up their own battle plans and reinforce a two-pronged strategy: Fight H5N1 to limit poultry losses and human exposure while also preparing for a pandemic.

So far, containing H5N1 has proved difficult. China reported new outbreaks last week, and many worry that, having reached Europe, the virus may next surface in Africa. Fighting bird flu there would be a logistical nightmare, says Modibo Traoré, head of the Interafrican Bureau for Animal Resources, an African Union agency. Surveillance in many countries is weak, diagnostic labs are underequipped, and there's no money to compensate farmers for culling their flocks. Meanwhile, the proximity between people and poultry would put many humans at risk, Traoré says.

The meeting produced consensus on a range of measures to prevent further spread of the virus and reduce the impact of a pandemic, from dispatching rapid response teams and strengthening lab capacity to expanding research on drugs and vaccines. The World Bank, which estimates that a pandemic could cost as much as $800 billion, said it hoped to set up a $1 billion fund for programs worldwide; the Asian Development Bank agreed to shell out up to $300 million on top of the $170 million already pledged. Individual countries promised to help out with expertise and money. (Concrete pledges are expected at a follow-up meeting in January in Beijing.)

But none of the proposals directly addressed the question of equitable access to medicines and vaccines should a pandemic strike. "Increasingly, our population is asking 'Why aren't we stockpiling' " the amounts of antiviral drugs ordered by Western countries, Malaysian delegation head Nor Shahidah Khairullah said at the meeting. "Drugs are expensive, and we live in poverty. How can we afford them?" adds Rachel Arungah, permanent secretary for special programs in the office of Kenyan President Mwai Kibaki.

The supply situation at least should improve, said WHO's Klaus Stöhr, reporting results from a meeting held with vaccine manufacturers a week earlier in Geneva. As many as eight companies are now developing pandemic vaccines; factoring in new formulations and delivery strategies, Stöhr said, in a couple of years the world might be able to produce 1.8 billion doses within 8 months of the start of a pandemic. But who would get them—apart from citizens of the countries where the companies are located—is unclear.

Switzerland-based Roche will make its popular flu antiviral Tamiflu available to developing countries for $12 per course of treatment instead of the $15 charged to wealthier nations, says a company spokesperson. But even with that discount, the price is out of reach for many developing nations. WHO flu chief Margaret Chan, who says she's "very sensitive" to the disparity, is currently negotiating with Roche about purchasing oseltamivir on their behalf. Chan declined to say how much WHO would purchase, for which countries, or at what price.

–MARTIN ENSERINK

CREDIT: LAURENT GILLIERON/KEYSTONE/AP PHOTO

ScienceScope

Cancer Genome Pilot Flies

A key advisory panel to the U.S. National Cancer Institute (NCI) has approved a controversial $100 million, 3-year pilot project to discover common gene mutations in human tumors.

The Human Cancer Genome Project could cost $1.5 billion over 10 years, with funding from NCI and the National Human Genome Research Institute. Some researchers have questioned the value of systematically sequencing tumors (*Science*, 21 October, p. 439). But this week, NCI's Board of Scientific Advisors endorsed the first two "requests for proposals," telling NCI to shift some money from sequencing to investigator-initiated grants and to set milestones for the full project. –JOCELYN KAISER

Report Says Plan B Decision Was "Unusual"

The Food and Drug Administration (FDA) followed an "unusual" review process in deciding not to permit over-the-counter sales of Plan B, also known as the morning-after pill, according to a report released this week by the investigative arm of the U.S. Congress. Several senior FDA scientists told investigators from the Government Accountability Office (GAO) that top FDA officials declared that the over-the-counter application would be rejected even before the reviews were completed. In a statement, FDA says the GAO report "mischaracterizes facts."

–JENNIFER COUZIN

Troubled Ottawa Appeals To Scientists

OTTAWA—Canada's ruling party is tempting researchers with a $1.5 billion basket of proposed investments over 5 years that it hopes will prove useful at the polls.

This week, Liberal Finance Minister Ralph Goodale unveiled a surprise minibudget that serves in effect as a campaign promise for an election likely to be held in January. If the Liberals win, scientists could see a modest boost for the three granting councils, a near doubling of support for university overhead, and more graduate scholarships. The budget also promises new efforts to commercialize university research. But Canadian Association of University Teachers Associate Executive Director David Robinson warns that "they missed the opportunity to deal with the real underlying issue: lack of core operating funding for universities."

–WAYNE KONDRO

NEUROSCIENCE

Neuroscientists Welcome Dalai Lama With Mostly Open Arms

A controversy over the Society for Neuroscience's (SfN's) decision to invite the Dalai Lama to its annual meeting faded last week when the Buddhist leader charmed an estimated audience of 14,000 in Washington, D.C., with a talk presenting meditative practice as an empirical way to investigate the mind and emphasizing his preference for scientific inquiry over religious dogma. His remarks were followed later in the meeting by a number of research presentations addressing whether meditation can alter brain physiology and offer health benefits.

More than 500 researchers, including many SfN members, had signed an online petition opposing the Dalai Lama invitation, arguing that it would blur the distinction between science and religion. And the furor took on a political element when neuroscientists supporting his invitation argued that the petition organizers were largely of Chinese ancestry and were trying to stifle recognition of Tibet's spiritual leader.

Open mind. Before reporters besieged him, the Dalai Lama told neuroscientists that they and meditators may have a lot to learn from each other.

But the only acts of protest at the meeting were the withdrawal of six posters from among thousands of submissions and a graduate student holding a sign that read "Dalai Lama not qualified to speak here," said SfN officials. The Dalai Lama's talk was the first in a series called Dialogues between Neuroscience and Society that SfN hopes will stimulate researchers to think more deeply about their roles in the larger world. "We thought he could draw our attention to the question of how compassionate behaviors can be developed," says SfN president Carol Barnes. (Celebrity architect Frank Gehry will be the speaker in the series next year.)

Calling for greater interaction between neuroscience and contemplative traditions, the Dalai Lama urged researchers to work toward human happiness by finding ways to reduce negative emotions and enhance positive ones. Judging from the laughter and applause that greeted some of his remarks, the talk itself seemed to have triggered a wave of good feeling. But some neuroscientists in the audience said the lecture didn't provide them with insights that could be useful to their field.

The Dalai Lama's presence did shine a spotlight on meditation research, which some scientists view as controversial because meditation is an integral part of many religions. Others see problems in the varying definitions of meditation and in the fact that scientists must rely on a meditator's claim of a subjective experience.

Nonetheless, Sara Lazar, a psychologist at Harvard Medical School in Boston, reported that she and her colleagues had found differences in brain structure between meditators and nonmeditators. Using magnetic resonance imaging scans, Lazar's group discovered that areas of the cortex associated with attention and sensory processing were thicker in subjects who had been practicing meditation for many years than in subjects with no meditation experience. "The differences in thickness were most pronounced in older subjects, suggesting that regular practice of meditation might reduce normal age-related thinning of the brain," Lazar says. This could, in theory, stem some of the cognitive decline typically seen with aging, she suggests.

In another study, Richard Davidson and his colleagues at the University of Wisconsin, Madison, examined the brain activity of six long-term practitioners of a type of meditation in which individuals attempt to generate compassion and kindness toward all by focusing their attention on an image or on their breathing. As they meditated, the subjects rated the intensity of their effort using a scaling arrow on a computer screen while the researchers recorded so-called gamma band rhythms in the subjects' brains using an electroencephalogram. The researchers found that the intensity of these impulses, which are associated with activities such as attention and learning, increased in correlation with the increase in intensity of the meditation effort. Davidson says the results show the possibility of tracking the activity of meditation through external means.

Experienced meditators such as the ones who participated in Davidson's study could help revive a tradition of introspective psychology, says neurologist Vilayanur Ramachandran of the University of California, San Diego. By asking them to describe internal experiences while meditating, it may be possible to figure out "fundamental laws of emotions, if there are any," he says. "As long as such studies are rigorous and subject to cross-subject verification, I don't see a problem."

Brian Knutson, a cognitive psychologist at Stanford University in Palo Alto, California, says the mental skills conferred by long-term practice of meditation could be invaluable in teasing out the neural mechanisms that underlie phenomena such as visual perception. "Some meditators claim to have the ability to slow down their cognitive processes," he says. "If that's true, one could in theory ask the subject to pinpoint different stages in the deconstruction and reconstruction of information that takes place during visual processing and discover the neural correlates for each of those steps."

Although receptive to using meditation as a scientific tool, some researchers questioned whether the Dalai Lama's talk added much on that issue. "He made some nice jokes," says Oliver Bosch, an empathy researcher at the University of Regensburg, Germany, referring to a remark by the monk that if researchers came up with a surgical technique to eliminate jealousy and hatred from the human mind, he'd be the first to sign up for it. "But he didn't offer any new ideas."

What the Dalai Lama may have offered is a plug for more funding for neuroscience. Humans spend "billions of dollars" exploring external space, he said, but not enough on probing their "inner space, where there are still a lot of things to explore." Few among the more than 33,000 people attending the SfN meeting would find that sentiment controversial.

–YUDHIJIT BHATTACHARJEE

CREDIT: KEVIN WOLF/AP PHOTO

TEACHING EVOLUTION

Antievolutionists Win One in Kansas, Lose Eight Seats in Dover

Both supporters and critics of teaching evolution in U.S. schools claimed victory last week in separate skirmishes. But when the dust had settled on the 8 November votes in Kansas and Pennsylvania, the only thing that was clear was that the battle will continue.

In Kansas, the state board of education voted 6–4 to adopt science standards that cast doubt on evolution (*Science*, 19 August, p. 1163). The action represents a repeat of a 1999 vote to introduce creationist ideas into the standards the last time they were modified. That team was kicked out the next year, but in 2002, creationists reclaimed a majority on the board. Supporters of evolution hope to rise again next fall in contests for five of the 10 seats.

In Dover, Pennsylvania, voters booted out eight school board members who supported intelligent design (ID). That vote came on the heels of a 6-week trial, *Kitzmiller* et al. v. *Dover Area School District*, in which parents challenged the board's decision to inform biology students about ID (*Science*, 16 September, p. 1796). With only one incumbent not up for reelection, the pro-evolution forces now count an 8–1 majority.

Lawyers defending the board had said previously they would fight an adverse ruling all the way to the U.S. Supreme Court. But now it appears they won't have a client. Several winners said before the election that they would not appeal if the district loses the case. But "the present thought is to wait for the judge's decision and go from there," says incoming board member Bernadette Reinking. A ruling is expected by early January.

Barbara Forrest, a philosophy professor at Southeastern Louisiana University in Hammond who testified for the plaintiffs, hopes the vote signals that "the pendulum is swinging back [toward science]." But biologist Karl Kleiner of nearby York College thinks the election "does not reflect a change in thinking on the part of the citizens of Dover" because the margin of victory was extremely narrow. "The bottom line is that nearly half of the community still feel that an alternate perspective to evolution should be presented to high school students," he notes. However, Kleiner also thinks residents are "going to agree to disagree on this issue" so that the community can disappear from the news.

The new board members say they don't assume the battle is over. "We will be preparing early for the next election [in 2007] because five seats will be available," says Reinking. The lawyers for ID advocates certainly are not prepared to admit defeat. Richard Thompson of the Thomas More Law Center in Ann Arbor, Michigan, who defended the Dover school board in the trial, called it "a watershed event. I think you're going to see ID popping up all over the country now," he predicted.

Back in Kansas, the board may not be able to move as quickly as it would like. That's because the U.S. National Academy of Sciences and the National Science Teachers Association have denied it permission to use language from their copyrighted publications in the new state standards (*Science*, 4 November, p. 754).

–CONSTANCE HOLDEN AND YUDHIJIT BHATTACHARJEE

Battling billboards. Dover is 70% Republican, but voters decided the "right" choice was a slate of pro-evolution candidates (*top*, *right*), all of whom ran as Democrats.

CREDITS (LEFT TO RIGHT): CAROLYN KASTER/AP PHOTO; ISAS/JAXA

ScienceScope

Rover Lost in Space

A tiny robotic rover intended to inspect the surface of the near-Earth asteroid Itokawa is drifting helplessly in space following a botched deployment. Dubbed Minerva, the rover was supposed to take images of the asteroid from which its parent craft, Hayabusa (shown as a shadow approaching Itokawa), will later collect rock samples. But a malfunction released Minerva 200 meters above the surface rather than the intended 60 meters, leaving it outside Itokawa's gravitational pull.

"It's really a shame," says Hayabusa project manager Jun'ichiro Kawaguchi of the Japan Aerospace Exploration Agency. Still, the agency hopes Hayabusa's descent close to the surface of Itokawa to release Minerva will pay off later this month when it attempts touchdowns to retrieve samples. Hayabusa is looking for clues about the composition of planetary bodies and how they have been transformed by "space weathering."

–DENNIS NORMILE

Lean Times for Nuclear Physics

U.S. nuclear physicists won't have enough money to run their two largest particle accelerators full-time. That's the bad news from Congress, which last week approved a 2006 budget for the Department of Energy's nuclear physics program that fell below earlier levels set separately by the House and Senate.

The Continuous Electron Beam Accelerator Facility at the Thomas Jefferson National Accelerator Facility in Newport News, Virginia, suffers a roughly $6 million shortfall, meaning fewer experiments than usual. The Relativistic Heavy Ion Collider at Brookhaven National Laboratory in Upton, New York, gets $17 million less than expected, meaning at most a 6-week run rather than the planned 20 to 29 weeks and possible layoffs of more than 100 employees, says BNL Associate Lab Director Samuel Aronson.

Physicists can weather one bad year, says Richard Casten of Yale University. But "if the '07 budget is like the '06 budget," he says, "it will be a disaster."

–ADRIAN CHO

New forecasts see a welcome easing of current tight oil supplies, but within a decade production outside OPEC will likely stall, they say, placing the burden on Middle East countries that may be unable or unwilling to respond fast enough

Bumpy Road Ahead For World's Oil

The oil business is nothing if not cyclical. Since 1859, when American Edwin Drake began drilling instead of digging for oil, petroleum has been boom or bust. Oil would gush from a newfound province such as east Texas, drillers would rush in, fortunes were made, oil markets became flooded, and prices plunged. But inevitably, the gushing would slow, rising demand would sop up the excess oil, and prices would rise, prompting fears of a permanent shortfall. Then the next big find—west Texas, Saudi Arabia, or the North Sea—would pop up and set off a new cycle. The pattern, however, will not continue much longer, say analysts.

Oil's golden age of discovery is ending, according to these forecasters. The world isn't about to run out of its favorite energy source for cars, trucks, and planes, but within a few decades it will begin to run short. In the most popular scenario, the oil cycle will probably go through one more familiar gyration as new drilling projects come online in the next few years, juicing up supplies and depressing the price of oil yet again. Then, according to forecasts by major oil companies and private consulting firms, the growth of oil production outside the 11 nations of the Organization of the Petroleum Exporting Countries (OPEC) will slow to a stop. Past 2015, OPEC, and especially four or five countries of the Middle East, will be left to slake the world's growing thirst for oil, currently running at almost 1000 gallons a second.

The prospect of a plateau in non-OPEC oil production only a decade away worries many observers. "The problem is we really don't know" the true reserves still in the ground in most OPEC countries, says petroleum analyst Michael Rodgers of PFC Energy, a consulting company in Washington, D.C. And even if the oil is there, importing countries have little more than a verbal promise that OPEC will make the Herculean effort to extract enough of that oil fast enough to meet growing demand. "We've got a real problem in 2 to 3 decades for oil," says geologist Thomas Ahlbrandt, who headed the U.S. Geological Survey's (USGS's) 2000 world oil assessment out of the Denver office. Coincidentally, 20 or 30 years is about how long it would take a determined United States to rein in its consumption and develop sufficient alternatives to crude oil (see sidebar, p. 1107).

More on the way? A dearth of non-OPEC oil may require more of Kuwaiti drillers within a decade.

Imminent doom?

High prices at the gas pump have made the fate of the world's oil supply a hot topic of late, but for aficionados, such concerns are not new. Some oil analysts—primarily geologists retired from major oil companies—have long been arguing that there isn't enough oil left in the planet to continue pumping out ever more barrels to meet the world's ever-growing demand (*Science*, 21 August 1998, p. 1128), which now stands at about 30 billion barrels a year.

These "peakists" have predicted that the world's total oil production would very soon reach a maximum and begin a sharp decline. Production from individual fields inevitably peaks, they note. Drillers punch into the biggest, easiest-to-produce pools of oil first and pump them out as fast as they can, at least if politics does not constrain the drillers. Production soars until pumping oil from the porous rock becomes a bit like trying to suck a sponge dry through a straw, and oil flow plunges. Whole provinces and even continents have behaved the same way, they note. Production from the lower 48 states of the United States peaked in 1970, as did the United States as a whole, and North Sea production peaked within the past few years, just 30 years after it began.

Peakists see the world oil peak coming within the next decade or so. The late M. King Hubbert of USGS observed that production of natural resources seems to reach a maximum when about half of all the resource that could ever be extracted has been produced. He then nailed the timing of the lower-48 peak 15 years before it occurred. Armed with production records and an estimate of the world's so-called ultimate recoverable resource, geologist Kenneth Deffeyes, a professor emeritus at

Princeton University, finds that the world peak will come before 2009. Leading peakist and retired oil company geologist Colin Campbell of Ballydehob in County Cork, Ireland, puts it before the end of this decade. Others say certainly by 2015 or 2020. The differences arise in part from the way different analysts emulate Hubbert's methods, but most stem from different numbers for the world's ultimate recoverable resource.

A brief sigh of relief

Oil production outlooks from a variety of organizations take a quite different view of the immediate future, at least, and oil geology has nothing to do with it. World outlooks from consultants PFC Energy and Cambridge Energy Research Associates (CERA) in Cambridge, Massachusetts, and from major oil companies such as ExxonMobil Corp. and Royal Dutch Shell generally begin by surveying what drilling projects both private and national oil companies have in the works. Ignoring anything as grand as ultimate recoverable resources, they look at individual projects already under construction, firmly planned projects, and known fields being evaluated for their production potential and likely to be developed. From such surveys, analysts estimate how much new world production will likely come on line in the next 5 to 8 years.

But from this added production, analysts must subtract how much less older, "mature" fields will be producing as they reach the descending side of the production peak. On balance, "global oil production capacity is actually set to increase dramatically over the rest of this decade," reported geologists Peter Jackson of CERA's London office and Robert Esser of the New York office in their June study. Other recent studies broadly concur. If deepwater projects such as those off Brazil and West Africa move ahead at all the way expected, says Rodgers of PFC Energy, the current tight supply situation could ease, oil supply would once again comfortably exceed demand, and oil prices would drop.

The coming squeeze

Project-by-project analysts may see an improved world supply in the short term, but as they look farther out, they see a possible problem. At 5 to 10 years in the future, additional factors loom large. The rate at which production declines in aging fields becomes particularly important outside OPEC, Rodgers says. Often, 3% per year has been cited as a typical depletion rate. But for particularly mature regions—those outside OPEC and the former Soviet Union (Russia and the Caspian Sea region)—production has not increased since 1998, he notes. Judging by the amount of capacity added since then to avoid any production decline, depletion rates in mature regions must be not 3% but 5% to 8% per year, says Rodgers.

In addition to depletion rates, analysts must estimate how much more oil than expected will be recovered from existing fields. Typically, only about 35% of the oil filling the cracks and pores of a reservoir can simply be pumped out. But advanced extraction techniques such as flooding the reservoir with water to push oil out can sometimes raise recovery rates to 50% and more. Drillers can also find more oil than initially assumed to be in and around a field by using increasingly sophisticated seismic imaging technology. And then analysts

If Not Cheap Oil ...

When the amount of oil being pumped around the world maxes out sometime in the next 30 years or so (see main text), we will need an alternative to tens of millions of barrels of oil per day. At an October workshop sponsored by the U.S. National Academies, though, experts on the leading alternatives made plain that even all the practicable substitutes combined won't be ready in 25 years to make up for a major shortfall.

Heavy oil—Some crude oil is too viscous to flow easily into a well on its own. Typically, pumping in steam "converts peanut butter into ketchup," said Robert Heinemann of Berry Petroleum Co. of Bakersfield, California. Currently, about 3 million barrels of heavy oil are produced per day. If the price is right, Heinemann said, heavy oil production might double in the next 10 years.

No help. If world oil peaks soon, technology such as this fuel-cell hybrid vehicle envisioned by Toyota won't be ready.

Oil sands—In Alberta, Canada, humongous steam shovels gouge out 100 tons of oily sand from the land at a time, eventually yielding 50 barrels of oil per shovelful. Steam injected into deep oil sand beds can also free up the oil for pumping. But the arduous and environmentally challenging extraction of oil from sand means that despite Alberta's abundant sands, only 3 million barrels per day may be produced in 2020, said Eddy Isaacs of the Alberta Energy Research Institute in Calgary.

Coal—Yes, coal could fuel your car. Friedrich Bergius proposed the first process for converting coal's big, heavy organic molecules into short chains of carbon and hydrogen in 1912. Germany fueled its Luftwaffe from coal during World War II. But David Gray of Mitretek Systems in Falls Church, Virginia, guessed that it would take oil prices consistently above $50 per barrel to get production from coal up to 4 million barrels per day by 2030.

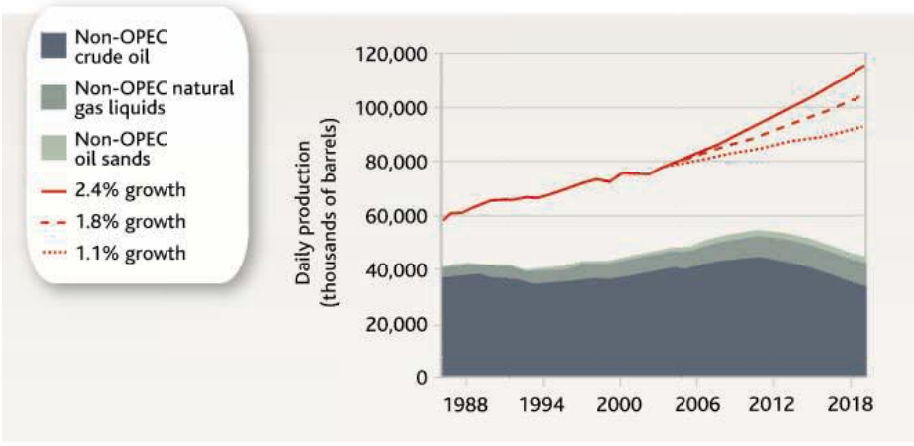
Natural gas—Trucks and buses already run on natural gas, but to ease international transportation of gas and to concentrate its energy, its single-carbon molecules can be chemically joined to form long-chain hydrocarbons, mostly a dieselike product. ExxonMobil is helping build a gas-to-liquids plant in the Persian Gulf nation of Qatar, Emil Jacobs of ExxonMobil in Annandale, New Jersey, said at the workshop. No other site has yet proven commercially viable. When pressed, Jacobs allowed that gas to liquids might yield half a million barrels of oil per day by 2015.

Conservation—John Heywood of the Massachusetts Institute of Technology in Cambridge noted that efficiency increases for U.S. cars have been entirely countered in the marketplace by the American predilection for bigger, heavier cars. And major steps up in efficiency with clean diesel engines and hybrids will take 30 years to have a substantial effect, he said, even under optimistic assumptions. Smaller cars will have to be in the mix, he concluded.

Nonstarters—Some energy sources will be of little or no use when the peak comes. Nuclear, wind, and solar do not produce liquid fuels. Liquids such as ethanol from biomass are not yet firmly economic. Oil from organic-rich shale won't be commercial for a decade or two, if then. Hydrogen for fuel cells would likely take half a century to have a substantial effect.

–R.A.K.

CREDIT: REUTERS



OPEC's challenge. Many forecasts have oil production outside of OPEC (solid-color bands) peaking in the next decade. That would require OPEC to fill the growing gap between non-OPEC production and world demand (red lines—dotted is low demand, dashed medium, and solid high).

must predict how many wholly new fields will be discovered, a procedure fraught with uncertainty.

Despite a range of methodologies, many production forecasts are now calling for a peak in the 2010s in oil production outside of OPEC. By 2015 or so, they indicate, non-OPEC producers—who supply 60% of the world's needs and boosted their output 35% during the past 25 years—will no longer be able to increase production. The ExxonMobil outlook, for example, has non-OPEC crude oil production reaching a plateau by 2010, holding steady for about a decade, and then declining. "Non-OPEC does plateau over time," says ExxonMobil's Scott Nauman of the Irving, Texas, office. "That's a reflection of the maturity of areas like the U.K. and the U.S." PFC Energy agrees. "Even if you make very optimistic assumptions," says David Greene of Oak Ridge National Laboratory in Tennessee, who has done such an analysis, "you come out with a[n] … oil peak outside of OPEC in the not-too-distant future."

The big one?

If more than half of the world's oil production is going to peak within a decade, "that has real implications for countries requiring huge imports to keep their economies running," says Rodgers. "Frankly, I think it's dangerous for the U.S. to bank on OPEC always being there to fill the gap." Just how dangerous a looming reliance on OPEC is depends on how soon you think OPEC's, and thus the world's, oil production is going to max out.

With the longer outlook comes greater uncertainty. Campbell has the OPEC and world peaks in this decade. While cautioning that the necessary data from OPEC countries are uncomfortably scarce, Rodgers and his PFC Energy team also calculate a relatively early OPEC/world peak. In part, they work from their observation that a country's production tends to peak and begin to decline when the total amount of oil ever produced from that country reaches 55% or so of all the oil yet reliably found there, called cumulative reserves. (This Hubbertian-sounding approach substitutes the more reliably determined cumulative reserves for ultimate recoverable resource.) Drawing on the available production and reserves data for OPEC countries, they find that—depending on how fast world demand for oil grows—OPEC and thus world production could peak as early as 2018 or as late as 2025.

Other analysts, perhaps most analysts, are more sanguine about OPEC's oil bounty. They generally argue that OPEC countries have not been exploiting their oil riches the way Americans have theirs, so OPEC production needn't behave like that of the United States. Ahlbrandt of USGS points out that, unlike North America, the Middle East is seriously underexplored. There are only 7000 wells in the whole region, he notes, a number equaled by the total wells in a few counties in a single U.S. oil basin. The 2000 USGS study he headed finds abundant OPEC oil—oil known to exist in reserves, likely to be found in and around existing fields, and likely to be discovered in new fields. Peakists, however, argue that some reserves are not as large as claimed and that additions to reserves from known fields will not be as large as they have been.

The latest studies by the U.S. Energy Information Administration (EIA) and by the Paris-based International Energy Agency combine the USGS numbers with expected price trends and with demand for oil, demand being a bit of a wildcard in any outlook. Both studies project rising world production out to 2025, which is as far as they looked. And using the field-by-field approach, the CERA study finds no OPEC peak before 2020, the farthest it looks, and the ExxonMobil study none before 2030. "There's no way we'll see a [world] peak in oil production for decades," says ExxonMobil's Nauman.

Assurances that the world will not soon run short of oil come with a caveat. OPEC countries may well have plenty of oil in the ground, but "we can't guarantee that the Saudis, the Iranians, and the Iraqis will spend sufficient funds and time to [ensure] demand will be met by growing supply," says Nauman. Presumably, OPEC countries will make the needed investments, the reasoning goes, or else they would lose oil sales to conservation, more expensive but more reliable sources such as Canadian oil sands, and alternative fuels.

OPEC certainly insists that it will come through for oil-consuming nations. OPEC acting secretary general Adnan Shihab-Eldin told a U.S. National Academies workshop last month in Washington, D.C., that OPEC will expand its production capacity to 38 million barrels per day by 2025, thus keeping supply "well above demand."

Far-future OPEC production is where politics and economics may prevail over geologic endowment. In its long-term projections, the U.S. EIA simply assumes that because OPEC countries have the oil, they will pump enough of it to fill the gap between future demand and non-OPEC capacity. In the case of Iraq, the latest EIA outlook has the Iraqi oil industry—now struggling to produce 2 million barrels a day—tripling its current production and achieving twice its highest previous production by 2025. At the same time, EIA concedes that OPEC countries would make more money in the long run by producing less than consuming countries demand but selling it at a higher price.

Too late already?

"We know a peak is coming," Robert Hirsch of SAIC Inc. in Arlington, Virginia, said at the academies workshop, "but we really don't know when." A peak a quarter-century away, however, would be uncomfortably soon for Hirsch. Peaks tend to sneak up on analysts, he notes. Even if a consensus on peak timing develops, "there will be no quick fixes," Hirsch found in a study he did for the U.S. Department of Energy this year.

Hirsch considered technologies for replacing crude oil that are ready or nearly ready for commercial use. He assumed 3 to 5 years to get crash programs up and going and optimistic rates of expansion of each program. Still, unless the crash programs were begun 20 years before the peak, shortages would occur. If they weren't begun until the peak arrived, "major shortages persisted a very long period of time," said Hirsch. "The downside of the optimists being wrong is dire."

–RICHARD A. KERR

SOURCE: PFC ENERGY

CHICAGO, ILLINOIS—A student-run meeting on evolution and development attracted top-notch researchers here from 20 to 23 October.

Hummingbirds Keep Plant Speciation Humming Along

Many evolutionary biologists argue that the extraordinary biodiversity in the tropics is the product of a long evolutionary history. But that's not the case for tropical plants called spiral gingers, says Douglas Schemske, an evolutionary ecologist at Michigan State University in East Lansing. Schemske and his colleagues have found that more than 50 distinct spiral ginger species have evolved from a single common ancestor in just a few million years. The researchers are building a strong case that, for these species, rapid evolution occurred as slight genetic changes altered the plants' ability to attract various bee species and, more important, hummingbirds. "This is a truly spectacular story," says Eric Haag, an evolutionary biologist at the University of Maryland, College Park.

Schemske's graduate student Kathleen Kay, now at the University of California, Santa Barbara, uncovered the spiral ginger story by analyzing DNA from 38 species of the plant across the globe. Based on the family tree she built, Kay concluded that the earliest spiral gingers hail from Africa. The modern representatives of these plants are pollinated by bees, suggesting that their ancestors were, too. Kay's data also show that one of these African ancestors wound up in the New World between 1.5 million and 7 million years ago.

During that time, conditions were ripe for plant—and animal—diversification. The newly forming Andes Mountains and other dramatic geological events provided new habitats, including cooler climes. Some of these new spots favored hummingbird pollinators, which do better in cold than bees, says Schemske.

The gingers themselves also had something to sell. Schemske's earlier work showed that hummingbirds often bypass bee-pollinated plants because the flowers have relatively little nectar. But spiral gingers have a lot of nectar, which likely prompted hummingbirds to take over for bees time and time again. Indeed, Kay's family tree places hummingbird-pollinated gingers on several branches, indicating multiple origins of this trait, she reports in the November *American Journal of Botany*.

It probably didn't take much more for spiral ginger species to pair up with specific bird species, Schemske adds. In experiments with another plant group, monkeyflowers, Schemske and his colleagues have discovered that slight changes in just a few genes alter the plant's flowers—a redder red, a more tubular blossom, etc.—and encourage distinct species of hummingbird pollinators. Spiral gingers show the same trends. As the plants adapt to new hummingbird pollinators, they no longer exchange genes with others that are pollinated by different birds, and one plant species splits into two. "In both [plant groups], pollinator specificity contributed to reproductive isolation," says evolutionary biologist Naomi Pierce of Harvard University.

Where hummingbirds hover. When bee-pollinated gingers lure in hummingbirds, new species can arise.

CREDIT: K. KAY/UNIVERSITY OF CALIFORNIA, SANTA BARBARA

Sometimes this reproductive isolation occurs without a change of pollinator, says Kay. Her unpublished data show that subtle differences in flower shape and size can result in pollen ending up on different parts of the bodies of a single hummingbird species, which in turn means that the pollen is transferred only to plants with a similar-shaped flower. In just a few million years, Kay concludes, such changes have resulted in the 50 or more New World spiral ginger species known today, which include 31 with hummingbird partners. "The diversification [was] at a rate comparable to the fastest known plant radiation," says Kay.

Evolutionary ecologist Thomas Juenger of the University of Texas, Austin, questions whether the spiral ginger speciation story is that simple. But, he acknowledges, "if it is simple to dramatically change characters that are important to pollinators, … then it may be much easier for reproductive isolation to evolve in plants than previously thought." That, adds Juenger, means diversity can arise much more quickly as well.

Development Out of Sync

In development, timing is everything. Get it wrong, and organs fail to grow or wind up in the wrong place. But for some animals, altering the normal sequence of organ formation can be key to survival—and, some evolutionary biologists argue, a driver of evolution. Take the case of the spadefoot toad.

These amphibians often lie buried in desert dirt for months, emerging within minutes of a rainstorm to hop to the nearest water to mate. Their tadpoles must then race through development before the pond dries up—in some cases, in little more than a week. Some species beat the clock not by speeding up growth overall but by accelerating changes in body parts critical to escaping before their watery world disappears, says Daniel Buchholz, a comparative endocrinologist at the National Institute of Child Health and Human Development in Bethesda, Maryland. In these toads, limbs and many organs develop faster than normal, but development of the gonads—which aren't needed until later in life and require developmental energy—isn't speeded up.

The key to this selective acceleration may be thyroid hormone, Buchholz reported at the meeting. Thyroid hormone is known to prompt spadefoot tadpoles to lose their tails and gills and punch out legs as they metamorphose into adults. Working with Tyrone Hayes, a comparative endocrinologist at the University of California, Berkeley, Buchholz measured thyroid hormone concentrations in the tail and liver of tadpoles from two spadefoot species, one that takes 33 days to metamorphose and another

that needs only about 14 days. The tissues of the earlier-metamorphosing species were two- to fivefold more sensitive to the hormone, he reported at the meeting. But there was no difference between the species in the rate of growth of the gonads, which do not respond to thyroid hormone. As a result, the 33-day tadpoles develop gonads before they metamorphose, whereas the 14-day tadpoles metamorphose and leave the pond before their gonads develop.

Christopher Rose, a developmental biologist at James Mason University in Harrisonburg, Virginia, says Buchholz hasn't completely nailed down the role of thyroid hormone. But he is impressed by the work. "He has [addressed] a developmental problem that is important not only to evolutionary biologists but ecologists as well," Rose says.

Spadefoot toads are not alone in speeding up the development of some body parts to gain an ecological advantage. Susan Hill, a developmental biologist at Michigan State University in East Lansing, reported at the meeting that at least one species of *Capitella*, a marine polychaete, develops adult musculature prematurely. Polychaete eggs typically hatch as larvae that swim and feed with the aid of bands of cilia. But a few species start out segmented like adults, with fewer bands of cilia and adult muscles throughout their bodies, says Hill. By staining and tagging proteins key to cilia or muscle development in one *Capitella* species, Hill and Barbara Boyer, a developmental biologist at Union College in Schenectady, New York, found that the cilia and muscles appear simultaneously rather than sequentially, as is usual for polychaetes. This accelerated development allows juveniles to put down roots fast when they come upon the right habitat, says Hill.

Quick-change artist. Some spadefoot toad tadpoles speed up metamorphosis, but gonad differentiation lags behind.

Both studies point to the value of asynchronous development in conferring survival advantages, says Rose. The phenomenon, he speculates, "has played a major role in the evolution of life history traits, morphological innovations, and possibly, body plans."

–ELIZABETH PENNISI

Profile Edward Ames

Uncovering the Hidden Paths Of Maine's Threatened Cod

Fifteen years ago, a collapse of the cod population brought Edward Ames back on land; now he is investigating ways to protect their spawning grounds

STONINGTON, MAINE—The Atlantic is shimmering under the October sun, beckoning Edward Ames to part from the shore. All his life he has heeded its call, as he does this afternoon, driving past waterside cafes and stores selling fishing gear to arrive at the harbor of this tiny fishing village. Climbing into his boat with his black lab Freckles, he motors toward the outer bay where he earns his living.

These are the hunting grounds that once provided Ames and other local fishermen with a rich harvest of cod and haddock. Then, about 20 years ago, the catch began to decline, forcing Ames and thousands of others to switch to lobstering. That is what Ames does all summer and fall, carting 400 lobster traps 5 to 8 kilometers offshore and returning several times a week to haul in the catch.

But he dreams of a day when these waters will once again teem with cod and haddock, providing an alternative source of income for fishing communities along the coast. In pursuit of this dream, Ames has become a scientist and conservationist. By analyzing anecdotal information collected from fishermen and combining it with scientific studies, he has identified migration patterns for subpopulations of cod and charted the species' decline in the Gulf of Maine since the 1920s. The work has earned him one of this year's $500,000 MacArthur fellowships and given him a platform from which to promote a small-scale, local approach to species conservation.

Ames's study of cod has aligned two big interests—science and fishing—that have tugged him in different directions. Thirty-four years ago, he earned a master's degree in biochemistry but shelved his academic aspirations and went to work at sea. Because Ames is trusted, researchers and locals say, he has helped give the science of fisheries management a credibility it lacked in the fishing world. And academics say the rigor of his work has established its value.

> **"Ted's different than a lot of scientists** because he has been catching fish for a living."
>
> —Dick Larrabee

"Ted's different than a lot of scientists because he has been catching fish for a living," says Dick Larrabee, a 28-year-old local fisherman. "Most scientists seem to work against us, but Ted really wants to help us get the fishing back." Dick Rice, an older fisherman who has known Ames for 40 years, says Ames "has helped many of us realize that in order for the fishing to survive, you've got to control it. Don't keep on just taking, taking, taking."

Anchored to the sea

Ames grew up in a seafaring family in Vinalhaven, an island 25 kilometers southwest of Stonington. His father, a captain on an otter trawler, and his grandfather, a retired lighthouse keeper, didn't think he was cut out for the business. "I was the scrawniest of the kids in the extended family—the runt of the litter," he says.

Heeding his grandfather's advice to avoid becoming "a barnacle on a rock" and seek out new challenges, Ames enrolled as a graduate student at the University of Maine, Orono. In 1971, after finishing his master's degree in biochemistry, he got a teaching job. But after 7 years he returned to the sea.

In the early 1980s, Ames roamed the Gulf of Maine in a tub trawler, taking in generous catches of cod, haddock, and other fish that feed near the ocean floor. But starting in mid-decade, the harvest began to decline. "The landings per tow were roughly halved each year. By the end of the decade, the catch for my vessel size had gone from 1000 to 100 pounds [450 to 45 kg] an hour. … When you saw 40 to 50 large industrial trawlers

CREDIT: WILLIAM FLAXINGTON

driving through the area, … you could figure out pretty quickly that the fishery was not going to last," says Ames, adding that smaller fishermen like himself were responsible too. "We went wherever we discovered a body of fish; once that would be depleted, we'd just move on to another."

Many small fishermen went out of business—including Ames, who sold his boat in 1990 and started a water testing lab. Shortly after that, the state set up a panel to find ways to rebuild groundfish stocks, appointing Ames a member. The panel's first task was to identify locations that had served as spawning habitats, with a goal of repopulating them. Ames became a researcher. The only way to get the information, he realized, was to interview retired fishermen: They would know of spawning grounds that perhaps no longer existed and had no need to keep secrets from competitors.

Working through the Maine Gillnetters Association, of which he was then executive director, Ames identified 28 former trawler captains who had been known "as the very best cod and haddock slayers" back in the 1930s and '40s. He asked them to list the places where they had found codfish ripe with eggs and sperm, an indication of a spawning area. Ames used two screening standards. He accepted a location only if it was independently identified by two or more interviewees, and he required that its geographical characteristics, derived from benthic maps, match the depth and seabed composition of typical cod spawning habitats. More than 75% of the data points survived this vetting process, providing a map of historical spawning grounds in the gulf. Ames overlaid this on a map from the 1920s, from which he could identify the areas where cod had been most abundant in different seasons.

By combining the two, Ames plotted the annual—and in some cases, seasonal—movement of fish away from and back to spawning areas. "I saw the neatest and most bewildering array of movements that I could have imagined," he says. It showed that groups of cod in different parts of the gulf were moving along unique pathways that often followed the contours of the coastal shelf and deeper offshore ridges. "There were separate and unique migration corridors that serviced particular spawning segments of the population but not others, which negated the perception that this was a mass body of fish that was shuffling back and forth in the gulf," he says. Comparing the map of historical spawning grounds to cod egg distribution studies conducted by the National Marine Fisheries Service in the late 1970s and '80s, Ames came up with another finding: Nearly half the spawning habitats that existed in the gulf in the 1930s have been abandoned. Ames plans to use some of the MacArthur money to study the historical relationship between populations of cod and alewives, a forage species for cod.

Ames's work suggests that policymakers need to look at the fine-scale structure of cod migration, says Joseph Wroblewski, a fisheries scientist at Memorial University of Newfoundland in St. John's, Canada, who has collaborated with Ames. Currently, fishermen may harvest cod and other groundfish anywhere in the gulf for a limited number of days every year. Ames and his supporters say that policy encourages large boats to pluck fish from wherever they find them, including inshore spawning habitats that are vital to rebuilding local fisheries. Instead, Ames says, regulators should protect nursery grounds from hard-bottom gear and prevent inshore fishing altogether during certain times of the year, while allowing fishermen relatively unrestricted access to the open sea.

To achieve those goals, Ames argues, "we need to introduce local stewardship to control how, when, and where we fish." With his wife Robin Alden, a former state marine resources commissioner, Ames has waged a campaign for local control through a nonprofit organization they founded called the Penobscot East Resource Center. "By empowering local governance units to manage local habitats, we could change the whole dynamic of conservation. The message it would send is that you have the right to make a living off this resource, but you are also responsible for taking care of it." He adds that such units would still have to work with state and federal enforcement agencies to protect their habitats from outside fishermen.

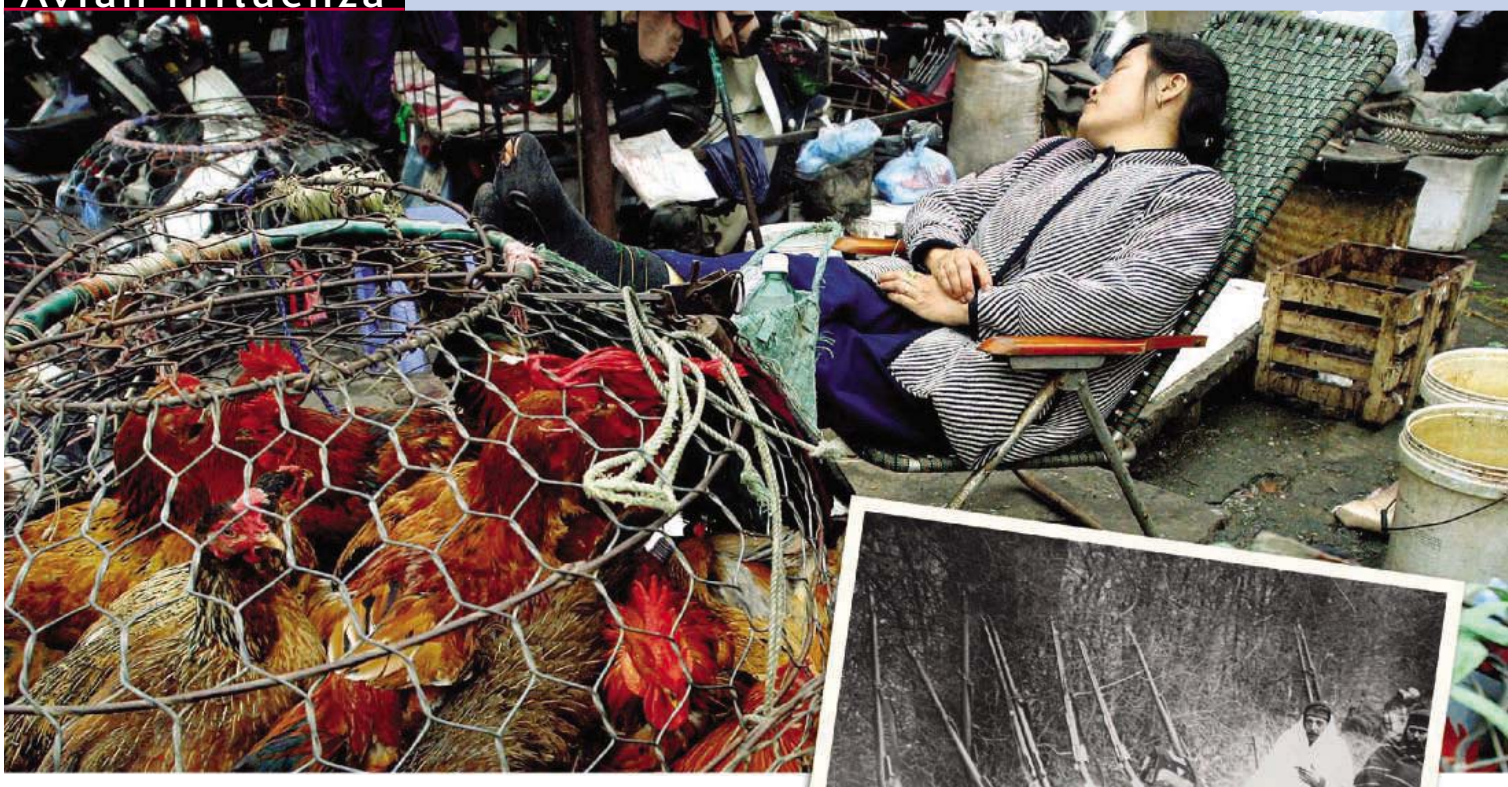
Ames has caught the attention of policymakers, says James Wilson, a fisheries economist at the University of Maine, Orono. "Five years ago, the reaction to him among federal and state officials was always negative: 'Who the hell is this guy; he's not a real scientist,' they would say. It's no longer that monolithic." Geoffrey Smith, a fisheries scientist at the Ocean Conservancy in Portland, thinks that Ames's ideas are having an impact. He says, "The New England Fisheries Council seems to be slowly moving toward developing management plans for smaller areas in the gulf."

Most important, some say, is that Ames's work is persuading fishermen that sustainability is a priority, and that scientific methods can help achieve that goal. In the past 2 years, lobsterers and businesses in Stonington have pooled $50,000 in a lobster hatchery that Ames is building. The facility is gearing up to culture 50,000 juvenile lobsters next year to release at select sites along the coastal shelf—a plan for which Ames intends to use a part of his MacArthur grant. It's a big investment. But, Ames says, as he digs his hands into his pockets and turns to the ocean, "it all comes back."

–YUDHIJIT BHATTACHARJEE

Hands-on. Fisherman-turned-researcher Edward Ames won a MacArthur grant after using seafarers' recollections to build a map of cod migratory routes.

CREDIT: COURTESY OF EDWARD AMES



Pandemic Skeptics Warn Against Crying Wolf

As flu fears mount, a number of scientists are questioning just how likely it is that the avian strain H5N1 will trigger a deadly human outbreak

Just as the threat of an influenza pandemic is finally being taken seriously by governments around the world, a small but increasingly visible number of scientists are questioning how great the danger really is. They acknowledge that another flu pandemic is inevitable—at least three major and several minor pandemics occurred in the last century—and they believe preparing for it is wise. But they are asking: Is the H5N1 virus now circulating in Asia really the one to watch? How soon will the next pandemic occur? And will it trigger a wave of mortality, as did the 1918 flu, or a just small ripple in the annual influenza death toll? If no serious pandemic emerges in the next few years, they warn, the current hype could backfire, undermining public support for efforts to prepare for an eventual pandemic, including developing and stockpiling better flu vaccines and drugs.

"I feel that there is some hysteria about H5N1," says Peter Palese, a virologist at Mount Sinai School of Medicine in New York City. Of course, he adds, no one can exclude the possibility of H5N1 touching off a pandemic, "but I don't think it is as much of a certainty as some of my colleagues make it seem."

Palese and other skeptics agree that H5N1 is a nasty virus that demands attention. The outbreak among poultry is raging out of control in Asia, where more than 150 million birds have been killed in futile attempts to control the disease since late 2003; the virus has now spread to Eastern Europe. Especially worrisome is its lethality for humans: 64 of the 125 people confirmed to have caught the virus in Asia have died. So far, however, the virus has proved very difficult to catch. With only one or two exceptions, human infections have resulted from close contact with diseased birds. But given how deadly the virus is, scientists and public health authorities have been warning that, if it mutates to become easily transmissible among humans, H5N1 could touch off a devastating pandemic. The World Health Organization (WHO) estimates that fatalities worldwide could range from 2 million to 7 million. And the longer and more widely the virus circulates, warn many scientists, the greater the odds that it will acquire such mutations.

Virus incubators. One hypothesis holds that the 1918 pandemic flu virus evolved in the trenches of World War I; some researchers worry that Asia's live animal markets could turn H5N1 into a human killer.

The skeptics are not convinced. "The virus is clearly not highly contagious among mammals, and I just don't think it's going to become so," says Paul Offit, an immunologist and virologist at Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine. Offit was also a member of the committee that advises the U.S. Centers for Disease Control and Prevention on vaccine policy. In a separate and more controversial argument, Paul Ewald, an evolutionary biologist at the University of Louisville, Kentucky, maintains that even if H5N1—or any other flu virus for that matter—acquires the ability to efficiently pass among humans, it is likely to fizzle into a mild form before killing very many people.

CREDITS (TOP TO BOTTOM): ASSOCIATED PRESS; HULTON-DEUTSCH COLLECTION/CORBIS

Historical solace

Offit bases his skepticism about H5N1 on two historical trends. He notes that no H5 flu subtype has ever caused a human pandemic. (Influenza viruses are subtyped by the forms of two surface glycoproteins: hemagglutinin [H] and neuraminidase [N]. Hemagglutinin is particularly important because it binds the virus to cells in the host's body.) Although human infections with H5, H7, and H9 subtypes have been documented, says Offit, these viruses have never been known to pass efficiently among humans. "That doesn't mean they never will, but there is some solace in the fact that they never have," he says. Palese and Offit both point out that although H5N1 has been circulating widely among poultry for at least 8 years, it has not shown any signs of jumping more easily from chickens to humans or of spreading among humans. "The virus has had ample time to mutate or reassort with genes from human influenza viruses, but nothing like this has happened," Palese says.

Buttressing his case, Offit points out that the six pandemics that have occurred since the late 1800s were caused by just three subtypes, which reappear in a repeating pattern: H2, H3, then H1. Roughly 68 years separated the reappearance of each subtype. He cites a hypothesis first proposed by the late Maurice Hilleman, a virologist who did pioneering flu research at the Walter Reed Army Institute of Research in Silver Spring, Maryland, and the Merck Institute for Vaccinology in West Point, Pennsylvania, in a 2002 paper in *Vaccine*. Hilleman speculated that 68 years is about the time necessary for most of those who were exposed to a pandemic subtype and developed immunity to die, leaving the world population "naïve" and thus susceptible to a pandemic of the same subtype. Based on this hypothesis, Offit reckons that the next pandemic will be caused by an H2 virus sometime around 2025.

Ewald maintains that any increase in transmissibility of H5N1 in humans will be associated with a massive drop in virulence, because killing the host is not a viable evolutionary strategy for a virus. He contends that the unprecedented mortality of the 1918 flu grew out of conditions in the trenches of World War I—a situation unlikely to be duplicated today, he says. During World War I, soldiers who were immobilized by illness were still able to come into contact with hundreds or thousands of others when they were carried to triage areas, then to field hospitals, and then packed onto crowded trains. (In a similar way, he thinks H5N1 became so highly lethal for chickens because of the intense crowding of modern poultry farms.) In typical conditions today, even in the crowded cities of Asia, Ewald maintains, seriously ill patients are confined to bed at home or in a hospital, where they come into contact with few others. Under these circumstances, Ewald believes that any virus newly introduced to humans would quickly evolve to low to moderate virulence. For these reasons, Ewald expects the next flu pandemic to be more like that of 1957, with 2 million deaths worldwide, or 1968, with 1 million.

War of words

Some scientists are particularly riled by Ewald's argument, which has received prominent attention since it was picked up by science writer Wendy Orent and reported in several articles in *The Los Angeles Times*, *The Washington Post*, and *The New Republic*. The articles have touched off flurries of commentaries, letters to editors, and attacks and counterattacks on Web logs. "She's treating Ewald's hypothesis as if it has as much experimental and theoretical support as the law of gravity," says Carl Bergstrom, an evolutionary biologist at the University of Washington, Seattle. "To the public, it is very misleading to represent one person's hypothesis as an accepted scientific fact."

Skeptics. Paul Ewald (*top*) thinks it would take conditions such as those at the Western Front to produce massively deadly pandemics. Paul Offit (*above*) finds solace in the fact that no H5-type virus has ever acquired the ability to pass easily among humans.

CREDITS (TOP TO BOTTOM): JOSHUA LUTZ/REDUX; CHILDREN'S HOSPITAL OF PHILADELPHIA

Marc Lipsitch, an epidemiologist at Harvard University who together with Bergstrom has taken to writing letters to editors who run Orent's articles, says the hypothesis about the trenches of World War I "is possibly true but far from proven." Lipsitch also notes that the crowded, filthy live animal markets of Asia have already been the source of one deadly human pathogen, SARS, and could well provide the conditions necessary to turn H5N1 into a pandemic virus.

And although the historical data are interesting, Lipsitch and others add, they simply aren't conclusive enough to rule an H5N1 pandemic in or out. "We don't know what viruses circulated in the past [among humans], except for the most recent 150 years," says Yoshihiro Kawaoka, a virologist at the University of Tokyo and the University of Wisconsin, Madison. What's more, he says, H5N1 is shattering historical precedents. Never before has a virus so highly lethal for poultry become so widespread and continued in circulation for such a long time. And with the virus continuing to spread, "the risk of mutation is increasing accordingly," says Masato Tashiro, director of WHO's Collaborative Center for Influenza Surveillance and Research at Japan's National Institute of Infectious Diseases in Tokyo. There are so many gaps in what is known about how virulence and pathogenicity evolve, Kawaoka says, that "there is no scientific basis to predict anything." Bergstrom agrees: "We, as scientists, need to do a good job of something slightly tricky here, which is to convey that our predictions are probabilistic."

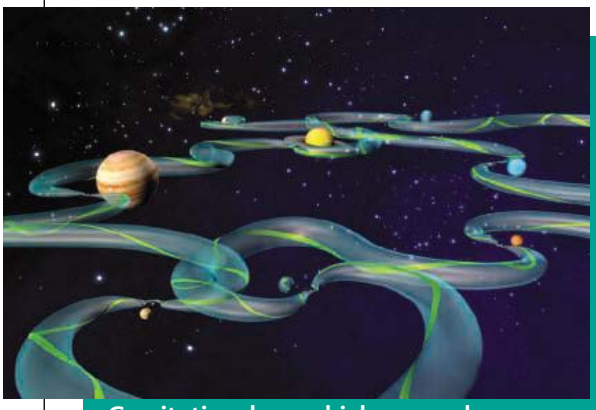
Despite their differences over H5N1, flu experts on both sides of the debate agree that preparing for a pandemic is essential (see p. 1103). Palese says he strongly supports the pandemic preparedness plan recently announced by the U.S. government. Ewald is in favor of tracking H5N1 and vaccinating exposed populations if the virus shows any tendency toward passing from human to human. "This could provide an effective barrier to evolutionary increases in transmissibility," he says. The plan is also similar to one of the strategies being pursued by WHO.

Offit hopes the concerns about H5N1 will lead to efforts to strengthen the U.S. infrastructure for vaccine development and production, which he says has deteriorated over the last 50 years. He thinks the message scientists should be sending "is not that we're going to protect you from the bird flu pandemic, but that we're going to be protecting you from a pandemic which may be 20 years from now."

–DENNIS NORMILE

Random Samples

Edited by Constance Holden



Gravitational superhighway snakes through the solar system.

Tube Route

Engineers compute spacecraft trajectories; quantum chemists track electron paths. Lately, both camps have found they're working on the same problems.

The recent 3-year Genesis mission, led by Martin Lo of the Jet Propulsion Laboratory (JPL) in Pasadena, California, and Kathleen Howell of Purdue University in Lafayette, Indiana, challenged engineers to find the best path for the spacecraft to leave Earth, sample the solar wind, and return. Genesis, with little fuel, had to navigate a gravitational obstacle course created by Earth, the moon, and the sun. Scientists devised an innovative route that took advantage of tubular, energy-efficient pathways, dubbed the "Interplanetary Superhighway" by Lo, that run throughout the solar system.

Charles Jaffe, a chemist at West Virginia University, Morgantown, noticed that Genesis's route bore an uncanny resemblance to the paths of ionized Rydberg electrons, which also follow tubular, low-energy pathways around protons. Inspired by that, Jaffe began collaborating with scientists at Caltech, JPL, and Georgia Tech, applying the techniques of statistical chemistry to plot asteroid movements through the solar system. Meanwhile, NASA has more ideas for using the Interplanetary Superhighway—including as a low-cost orbit for a space station between Earth and the moon. The math behind the Genesis trajectory is described in last month's *Notices of the American Mathematical Society*. "It may open new doors to people who study planetary mechanics," says Shane Ross, a dynamicist at the University of Southern California in Los Angeles. "And what we do may help chemists solve their problems as well."

Monkey Struggle In Wisconsin

A court case centering on the meaning of "consideration" may determine whether the University of Wisconsin, Madison, can prevent the construction of an animal-rights exhibit in the midst of its primate research facilities.

Last year, Rick Bogle, head of the Primate Freedom Project, negotiated an option to buy a small plot located next door to the Wisconsin National Primate Research Center. His project wants to use the land for an exhibit hall "illuminating the inhumane practice of using primates" in research. Owner Roger Charly agreed to sell for $675,000, further agreeing that the "consideration" for the option was "adequate" even though no money changed hands.

Last summer, the university got wind of the deal and offered Charly $1 million. Charly told the activists he would rescind their option unless they offered some tangible consideration. Last month, after learning that Charly had accepted the university's offer, the activists sued for breach of contract and on 26 October asked Dane County Circuit Court for a temporary injunction against the sale.

The activists' lawyer, Kendall Harrison of LaFollette, Godfrey, and Kahn in Madison, says, "The contract says consideration was sufficient." Charly's lawyer, Jon Manzo, says the option is void because "simply acknowledging consideration doesn't make it exist. My guy was receiving no benefit." Alan R. Fish, associate vice chancellor of the university, says it has paid Charly $1000 for the option and that it plans to use the land to expand its primate facility.

The Nonattachment Hormone

In 95% of mammalian species, males never bond with mates or help raise young. So what makes men inclined to roost and nurture? Testosterone seems to play a role.

Although high levels are associated with aggressive behavior in animals, they plummet with parenting in some species. Studies in North American males suggest the same trend in humans. Now comes evidence from China that this holds true regardless of culture. A team led by Peter Gray, a biological anthropologist at the University of Nevada, Las Vegas, recruited 66 bachelors, 30 married men without children, and 30 married fathers aged 21 to 38 in Beijing who twice a day provided saliva for testing. Compared with bachelors, childless husbands had about 20% lower levels of the hormone in the morning (when levels are highest), and married fathers had almost 50% lower levels. Smaller but significant differences showed up in afternoon measurements, the team reported last week in *Proceedings of the Royal Society: B*.

Low-T dad?

Psychologist Nick Neave of Northumbria University in Newcastle-upon-Tyne says the findings make sense because testosterone is related to "a host of sexual behaviors intended to attract a mate [but which] are not conducive to marital bliss and especially not when very young children are present." He adds: "It would be interesting to see if testosterone levels are associated with poorer male parenting skills."

Record-Setter No More

After 5 years of being the world's largest free-floating object, the 115-kilometer-long B-15A iceberg, tracked by the European Space Agency's Envisat satellite, broke up late last month off Antarctica's Cape Adare.

CREDITS (TOP TO BOTTOM): JPL/CICI KOENIG; ESA/R. STONE

Edited by Yudhijit Bhattacharjee

ON CAMPUS

Hiring woes. The second-highest-ranking administrator in the University of California system has resigned amid charges that she helped her son and a business partner get university jobs.

M. R. C. Greenwood, who has been provost of the University of California (UC) system since 2004, is being investigated for her role in hiring a friend, Lynda Goff, first as a faculty associate and later as director of UC's Science and Math Initiative. "It has been disclosed, in the wake of inquiries by the *San Francisco Chronicle*, that Provost Greenwood and Dr. Goff have until recently had jointly owned rental property," UC president Robert Dynes said in a 4 November statement. He said Greenwood would return to her job as a biology professor at UC Santa Cruz, where she was chancellor for 8 years before taking the UC post. "It appears that Provost Greenwood may have been involved in Dr. Goff's hiring to a greater extent than was appropriate, given that her business investment with Dr. Goff had not been properly and fully resolved in accordance with conflict of interest requirements," the statement said. UC is also looking into Greenwood's role in the hiring of her son James Greenwood as a paid senior intern on the new UC Merced campus.

Science was unable to reach Greenwood for comment. Dynes's statement clarifies that "there is no presumption of wrongdoing" and that the university expects to conclude its investigation "shortly."

MOVERS

Follow the money. Singapore has snagged a power couple from the U.S. National Cancer Institute (NCI) to help staff the country's $2 billion investment in biomedical research. Cancer geneticists Neal Copeland, 58, and Nancy Jenkins, 55, are leaving NCI next year after 2 decades to join Singapore's Institute of Molecular and Cell Biology. Their 18,000 research mice are coming, too.

Copeland and Jenkins run NCI's Mouse Cancer Genetics Program and have co-authored nearly 700 papers based on mouse models of human diseases. Copeland says the couple began looking around because of declining intramural research budgets at NCI and a ban on consulting for outside companies. Singapore, which wants to expand its biotech industry, will impose no such restrictions. They have also become fond of Asia through traveling there, he says.

"In Singapore, the money seems to be good right now," says Copeland. "It's just a great opportunity and a really interesting part of the world."

AWARDS

Cancer prize. A trio of young life scientists will share a $150,000 prize for helping unravel the molecular signals driving cancer. The Paul Marks Prize for Cancer Research, founded in 2001 and awarded every other year by Memorial Sloan-Kettering Cancer Center in New York City, recognizes scientists age 45 or younger. This year's winners are Tyler Jacks, 44, of the Massachusetts Institute of Technology in Cambridge, who builds and learns from novel mouse models of cancer; Scott Lowe, 41, of Cold Spring Harbor Laboratory in New York, an expert in cell death and aging; and Jeff Wrana, 44, of the University of Toronto in Canada, who is deciphering signaling pathways in cancerous and normal cells.

Got any tips for this page?
E-mail people@aaas.org

THREE Q's

In January, cognitive neuroscientist **Michael Gazzaniga** will leave the snows of New Hampshire (and an endowed chair at Dartmouth College) to head the new SAGE Center for the Study of the Mind at the University of California, Santa Barbara. He was in California last week for the center's launch.

Q: Honestly now, was it the beaches that drew you to Santa Barbara?

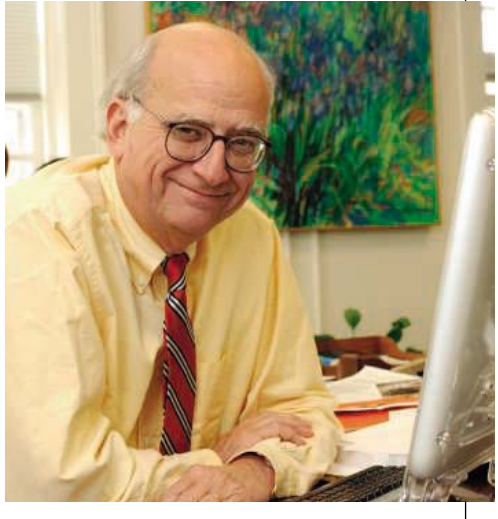
A: [Laughs] It's sunny and beautiful here today! The professional reason was the opportunity to get a true conversation going between social scientists and neuroscientists on the mind.

Q: Do the humanities types know enough about the brain to keep up their end of the dialogue?

A: Well, you do have to pick the right people. In the past few years, you see almost a wild interest in social psychologists coming into the study of the mind. What the mind does all day is think about social interactions. We don't sit around thinking about word lists or phone numbers. The social scientists can help neuroscientists think about more complex issues.

Q: Like neuroethics? Your longtime friend, newspaper columnist William Safire, coined the term. Do you have to pay him royalties each time you use it?

A: No, but I'm working on my next book, about what makes humans unique, and he just gave me a great title. But I can't tell you what it is.



CREDITS (MIDDLE, BOTTOM): UNIVERSITY OF CALIFORNIA; JOSEPH MEHLING/DARTMOUTH COLLEGE

New Directions in Plastic Debris

THE LARGEST EVER MEETING FOCUSING ON plastic debris in the environment was recently held in Redondo Beach, California (*1*). It is evident that plastic waste presents major concerns in aquatic habitats worldwide. However, this meeting differed from previous efforts/gatherings because representatives from industry, government, academia, and nongovernment organizations were united in their desire to identify solutions to reducing waste. There has been a switch in the types of litter recorded, from shipping- and fishing-related debris to land-based sources. This was poignantly underscored by reports of islands of plastic debris swept into the sea by Hurricane Katrina.

Polymer scientist A. Andrady explained that all the plastic introduced into the oceans remains unmineralized as either entire objects or fragments, some of which are less than 20 μm in diameter (*2*). Large items of debris cause entanglement, impaired feeding, and mortality to birds, turtles, and mammals. Microscopic fragments are also ingested, but the consequences are unknown. H. Takada and C. Moore presented evidence on the ability of plastic to accumulate PCBs, DDE, and nonylphenol (*3*), and the potential for toxic chemicals to transfer to the food chain was identified as a key research direction. It was also recognized that better understanding of effects at an organismal level is required before consequences at population and ecosystem levels can be examined.

In terms of solutions, much could be achieved by reductions in packaging. Keynote speaker W. McDonough made the case for a "cradle to cradle" (*4*) strategy to ensure that plastics are retained in a product-specific recycling loop—turning debris from a waste disposal liability into feedstock for production. Although debris can be removed from drains and rivers by physical separators, there is also a key role for education to help reduce littering. The importance of social research to establish the public's willingness to engage with these solutions was also clearly recognized.

RICHARD THOMPSON,[1]* CHARLES MOORE,[2] ANTHONY ANDRADY,[3] MURRAY GREGORY,[4] HIDESHIGE TAKADA,[5] STEPHEN WEISBERG[6]

[1]School of Biological Sciences, University of Plymouth, Plymouth, Devon PL4 8AA, UK. [2]Algalita Marine Research Foundation, 148 North Marina Drive, Long Beach, CA 90803, USA. [3]Research Triangle Institute, 3040 Cornwallis Road, Research Triangle Park, NC 27709–2194, USA. [4]University of Auckland, Private Bag 92019, Auckland, New Zealand. [5]Tokyo University of Agriculture and Technology, Tokyo 183 8509, Japan. [6]Southern California Coastal Research Project, 7171 Fenwick Lane, Westminster, CA 92683, USA.

*To whom correspondence should be addressed. E-mail: r.c.thompson@plymouth.ac.uk

CREDIT: DAVID QUINN/AP PHOTO

Toy cars amid debris in New Orleans after Hurricane Katrina.

References

1. Plastic Debris Rivers to Seas, organized by the California Coastal Commission, 7 to 9 Sept 2005.
2. R. C. Thompson *et al.*, *Science* **304**, 838 (2004).
3. Y. Mato *et al.*, *Environ. Sci. Technol.* **35**, 318 (2001).
4. W. McDonough, M. Braungart, *Cradle to Cradle* (North Point Press, New York, 2002).

Preparing for the Worst-Case Scenario

THE REPORT "CONTAINING PANDEMIC influenza at the source" by I. M. Longini Jr. *et al.* (12 Aug., p. 1083) was encouraging that an avian pandemic can be contained if proper intervention is carried out promptly. N. M. Ferguson *et al.* published similar findings (*1*). However, further investigation is needed before we can celebrate.

A valid conclusion from a model requires a careful selection of the parameter values. Longini's article assumed that Tamiflu (oseltamivir) was useful in a pandemic. Yet, Tamiflu may not be effective on all new avian flu viruses, which can have 80% mortality; Tamiflu was ineffective in 50% of patients in Thailand (*2*). Moreover, although the basic reproduction number (R_0) below 2 was a reasonable estimation in Longini's models, previous flu pandemics have had an R_0 up to 3 (*3*). Apart from these technical parameters in a hypothetical model, the logistics in a real situation can also be fluid. Although developing countries with pharmaceutical factories can issue a compulsory license to make generic copies of patented drugs in the event of a medical emergency, in reality when there is a pandemic occurring, developing areas without such facilities (perhaps those that most need Tamiflu) will be in a difficult position to secure enough supply.

Handling the ever-changing disease pattern of pandemic avian flu requires a contingency plan to prepare for the worst scenario. A worst-case scenario model should predict the resources a public health system needs to cope with a pandemic. This model should consider that Tamiflu may be ineffective in treating the new strain, a higher-than-expected R_0 value, and the possibility of a paucity of antiviral drug/vaccines. Such a worst-case-scenario model provides valuable information for resource planning, for example, the number of ventilators, the amount of intensive care, and even funeral facilities that will be required.

PUI HONG ALEX CHUNG

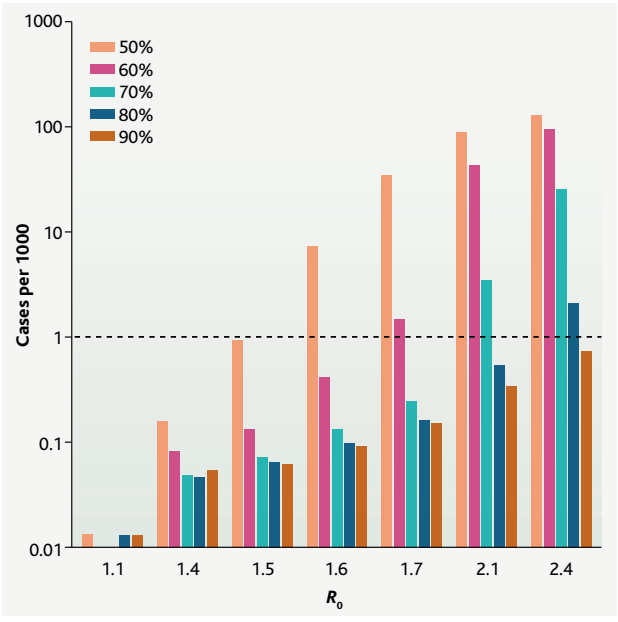
Higher Specialist Trainee in Community Medicine, 10th floor, 147C Argyle Street, Mong Kok, Hong Kong SAR, China.

References

1. N. M. Ferguson *et al.*, *Nature* **437**, 209 (2005).
2. T. H. Tran *et al.*, *N. Engl. J. Med.* **350**, 1179 (2004).
3. B. Cooper *et al.*, poster presented at Influenza Vaccines for the World, Lisbon, 24 to 26 May 2005.

Response

CONTAINING A POTENTIAL STRAIN OF PANdemic influenza at the source will not be easy and will require intense surveillance of influenza-like illness, speedy lab work to identify isolated viruses, and feasible and effective control strategies. We showed that the targeted use of oseltamivir could contain a potential pandemic strain of influenza if the basic reproductive number (R_0) were below 1.6 and if the intervention took place 2 to 3 weeks after the first case appeared. If the efficacy of oseltamivir against the



The effectiveness of the household and neighborhood cluster quarantine started 14 days after the first case at different values of R_0. Outbreaks that result in a cumulative incidence of ≤ 1 case per 1000 are considered contained (horizontal line). The cumulative incidence per 1000 does not always decrease monotonically with increasing intervention coverage for small differences due to stochastic variability.

emergent virus were as low as 50% against illness and transmission, the targeted use of oseltamivir could still effectively contain the spread (figs. S16 to S18). For a situation in which oseltamivir is ineffective against the emergent strain, we modeled the use of voluntary household and neighborhood cluster (i.e., one small grouping of about four households) quarantine alone (figs. 3 and S13). For this intervention, the first case in a locality (i.e., a region roughly 6 to 9 km in radius) triggers a quarantine policy. Every case and a certain percentage of susceptible people restrict their movement to their household and their neighborhood cluster. The figure gives the average simulated cumulative influenza case incidence for different levels of quarantine effectiveness for different values of R_0. For $R_0 \leq 1.5$, quarantine levels as low as 50% of people being restricted to the household and neighborhood cluster could possibly contain outbreaks. For $R_0 \leq 1.6$, quarantine levels of 60% or higher could be effective. With an R_0 as high as 2.4, we would need a 90% effective quarantine for containment. For larger values of R_0, containment would not be possible with quarantine measures alone. If we consider an R_0 of 2.4 and total viral resistance to oseltamivir to be the worst-case scenario, then only an extremely tight household and neighborhood cluster quarantine could effectively contain the spread of the virus. Other forms of quarantine also could be effective (*1*). Our results are highly probabilistic, and a strategy that works in many simulations does not necessarily work in all simulations. Thus, even if countries are prepared to try to contain the outbreak, the world needs to be prepared for the event that containment fails.

It is important to develop international cooperation around the maintenance of a mobile stockpile of oseltamivir that can be rapidly deployed to the location of an emergent strain. The World Health Organization (WHO) is currently developing such a stockpile and recently announced that Roche is donating 3 million courses of oseltamivir to its mobile stockpile (*2*). If a potential pandemic strain of influenza emerges, WHO plans to intervene, in concert with the ministry of health of the affected country and other public health organizations, along the lines that we describe above [see index 1 of (*2*)]. Such international cooperation coupled with a rapid, effective response will be necessary to have any hope of containing pandemic influenza before it spreads throughout the world, or at least slowing the spread while a well-matched vaccine is developed and sufficient quantities are deployed.

IRA M. LONGINI JR. AND M. ELIZABETH HALLORAN
Department of Biostatistics, The Rollins School of Public Health, Emory University, 1518 Clifton Road, NE, Atlanta, GA 30322, USA.

References
1. N. M. Ferguson *et al.*, *Nature* **437**, 209 (2005).
2. "Donation of three million treatments of oseltamivir to WHO will help early response to an emerging influenza pandemic" (available at www.who.int/mediacentre/news/releases/2005/pr36/en/), accessed 30 Aug. 2005.

Timing in Collection of Stool Samples

WE READ WITH GREAT INTEREST THE REPORT "Diversity of the human intestinal microbial flora" by P. B. Eckburg *et al.* (10 June, p. 1635; published online 14 Apr.). We applaud the authors for advancing this important field by undertaking comprehensive 16S rDNA sequencing to describe the composition of the microbiota in stools and at six sites of the colon in each of three human volunteers. The analyzed 13,355 prokaryotic ribosomal RNA gene sequences represent the largest 16S rDNA microflora data set reported to date in any species.

On the basis of their analysis, the authors suggest that "differences between stool and mucosa community composition" exist. We question the validity of this conclusion, based on our own observation that stool microflora composition can vary significantly in stool samples collected before and after a colonoscopy (*1*). The authors compare microflora composition in colon biopsy samples obtained during colonoscopy with a stool sample collected a month afterwards. The authors acknowledge potential problems with their interpretation because of the delayed stool collection, but a rationale for collecting delayed stool samples is not given.

This Report has significantly expanded our knowledge of the diversity of the intestinal microflora in a few subjects. However, if we ever want to correlate microflora composition with health or disease, we will have to design studies aimed at understanding the variation in the microflora composition in a large cohort of human subjects.

VOLKER MAI,* O. COLIN STINE, J. GLENN MORRIS JR.
Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland, Baltimore, MD 21201, USA.
*To whom correspondence should be addressed. E-mail: vmai@epi.umaryland.edu

Reference
1. V. Mai, O. C. Stine, J. G. Morris Jr., unpublished data.

Response

OUR LARGE-SCALE COMPARATIVE ANALYSIS of bacterial and archaeal 16S rDNA sequences in the colon and stool revealed significant intersubject variability and patchy heterogeneity among the colonic mucosal bacterial populations. Regarding the statistical differences we reported between stool and adherent mucosal populations, subjects B and C harbored different bacterial populations in their colonic mucosa compared with their stool samples collected 4 weeks later, while the mucosal populations in subject A were subsets of the population observed in stool collected 4 weeks after colonoscopy. Each subject's stool community was more similar to the communities of their own mucosal samples than to any community from a different subject.

We acknowledged that the statistically significant difference between the bacterial composition of the stool and colonic mucosa may have been due to the 4-week delay in stool collection after colonoscopy. The collection of stool was not originally planned in the large Canadian population-based case control study from which the control subjects were selected. For this

study of three healthy subjects, from whom the colonic tissue biopsies had already been collected, we chose to obtain stool samples 1 month after colonoscopy when each subject had full recovery of stable, baseline bowel function. Despite the original study design, we agree with Mai *et al.* that stool samples before endoscopy may provide more reliable representations of the steady-state stool bacterial population. However, a controlled comparative study is needed to reveal the degree to which stools are microbiologically dissimilar at various time intervals before and after bowel cleansing. A small study using denaturing gradient gel electrophoresis has suggested that the bacterial composition in colonic mucosal biopsies differs significantly from that in stool collected prior to the procedure (*1*), supporting our conclusions that significant differences exist between these microbial communities. Future studies should address these issues with high-resolution molecular methods and a greater number of subjects.

PAUL B. ECKBURG,[1] ELISABETH M. BIK,[2] CHARLES N. BERNSTEIN,[3] LES DETHLEFSEN,[2] ELIZABETH PURDOM,[4] MICHAEL SARGENT,[3] STEVEN R. GILL,[5] KAREN E. NELSON,[5] DAVID A. RELMAN[6]

[1]Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA 94305–5107, USA. [2]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305–5124, USA. [3]Section of Gastroenterology, University of Manitoba, Winnipeg, MB R3A 1R9, Canada. [4]Department of Statistics, Stanford University, Stanford, CA 94305, USA. [5]The Institute for Genomic Research, Rockville, MD 20850, USA. [6]Departments of Medicine, and of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305–5124, USA, and Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304, USA.

Reference

1. E. G. Zoetendal *et al.*, *Appl. Environ. Microbiol.* **68**, 3401 (2002).

CORRECTIONS AND CLARIFICATIONS

Editors' Choice: "Grabbing hydrogen" (4 Nov., p. 749). The item should have described the activation of C-H bonds as typically proceeding by oxidative addition of a C-H bond to a low-valent metal complex.

Policy Forum: "Proof of safety at Yucca Mountain" by L. J. Carter and T. H. Pigford (21 Oct., p. 447). The figure parts should have been reversed. The original design is shown on the right and the capillary barrier design is shown on the left.

Brevia: "Bacterial immunity traded for sperm viability in male crickets" by L. W. Simmons and B. Roberts (23 Sept., p. 2031). The URL for the Supporting Online Material was omitted. The correct URL is www.sciencemag.org/cgi/content/full/309/5743/2031/DC1.

Reports: "A chromium terephthalate–based solid with unusually large pore volumes and surface area" by G. Férey *et al.* (23 Sept., p. 2040). The crystal structure deposition number for **MIL-101** was omitted; the CSD number is 415697. There were errors in note (*23*); it should read "Initial hydrogen storage measurement was 4.5 weight percent at 77 K at 3 MPa. This value seems to be the highest for MOFs after the contestation of previous results (*3*)." Finally, the last sentence of the Fig. 1 legend should read "Chromium octahedra, oxygen, fluorine, and carbon atoms are in green, red, red, and blue, respectively."

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

NEUROSCIENCE

Nerves as Chemical Messengers

Arvid Carlsson

During the greater part of the previous century, neuroscience was dominated by neurophysiology. Electrical stimulation and recording of the activity of nerve cells contributed much to our understanding of nerve function, and this further strengthened the prestige of neurophysiology. But there were some early indications that not everything could be explained by electrical signaling. More than a century ago, some pharmacologists reported that certain drugs caused effects on blood pressure, heart rate, and so on that were very similar to the changes induced by electrical stimulation of the autonomic nervous system. The hypothesis was put forward that endogenous chemical compounds similar to these drugs existed and were released from nerve endings to trigger the response. In the early 1920s, an ingenious experiment on the frog heart by the German pharmacologist Otto Loewi (which showed that the vagus nerve secretes acetylcholine) provided the first proof of this hypothesis. Subsequently, a number of skillful pharmacologists, including Henry Dale in London, demonstrated chemical transmission in various parts of the mammalian peripheral nervous system. Loewi and Dale shared the Nobel Prize in 1936. Through the 1930s and into the 1950s, the pharmacologists' interpretation that these effects were due to a chemical signal was vigorously attacked by the neurorophysiologists, headed by the Australian John Eccles. This clash of opposing explanations, the "war between the soups and the sparks," finally ended with a victory for the soups.

Honored during the debate. Dale and Loewi won their Nobel Prize in 1936, but the controversy over how neurons communicate continued for another 15 years.

In *The War of the Soups and the Sparks*, Elliot Valenstein rightly remarks that no advances in brain research during the past 50 years have had a greater impact on our ideas about the brain than the discovery that the nerves secrete neurotransmitters when communicating with other nerves and cells they innervate. Valenstein's masterful account of this development fills a serious gap in the literature. The book will certainly be enjoyed not only by the educated public but also by scientists—and not least by those actually working in the field. It shows that the author (an emeritus professor of neuroscience at the University of Michigan) has perused an enormous scientific literature. In addition, Valenstein offers fascinating accounts of the lives of several of the key people involved in the discovery. He rightly emphasizes the researchers' markedly different personalities, which probably helped to speed the discoveries. Many of them communicated with each other in a mostly friendly and fruitful way. Even Dale and Eccles, the two fiercest combatants, developed great respect for each other. That is evident, albeit mingled with subtle irony, in the published correspondence between them, which extends over a quarter century.

In Valenstein's story, like so many others, it is remarkable how much resistance truly revolutionary discoveries often meet. That resistance, reflected in the book's title, shows up (in a sometimes dramatic manner) at every step of this complex discovery process. Loewi struggled for several years to gain acceptance of his findings. To convince the scientific community, he personally demonstrated his crucial experiment at an international physiology congress in 1926, and even after this success several researchers expressed serious doubts. After the novel discoveries were finally accepted within the scientific community, it took another several years (sometimes more than a decade) until they entered the standard physiology textbooks.

The reviewer, who shared the 2000 Nobel Prize in Physiology or Medicine for work on signal transduction in the central nervous system, is at Thorild Wulffsgatan 50, S-413 19 Gothenburg, Sweden. E-mail: arvid.carlsson@pharm.gu.se

The War of the Soups and the Sparks
The Discovery of Neurotransmitters and the Dispute over How Nerves Communicate
by Elliot S. Valenstein

Columbia University Press, New York, 2005. 255 pp. $31, £19.50. ISBN 0-231-13588-2.

As Valenstein mentions, I witnessed considerable resistance myself, when at a 1960 meeting in London I advocated a role for dopamine and norepinephrine as brain neurotransmitters. Surprisingly, this opposition was expressed by some of the major proponents of chemical transmission in the peripheral nervous system, headed by Dale himself. These pioneers apparently felt considerable hesitation when it came to the question of chemical transmission in the central nervous system. Perhaps the vigorous debate between the soups and the sparks not so many years earlier had made them especially cautious regarding the central nervous system. Their reaction may also have reflected the fact that so many seemingly revolutionary scientific discoveries turn out to have a short life span, thus providing good reason for skepticism.

A dramatic interval in the scientific developments described in the book coincided with an equally dramatic moment in 20th-century political history. Several German scientists played a decisive role, but they did so mainly as refugees in the United Kingdom after being expelled from their country by the Nazi regime. (Loewi, who fled from Austria in 1938, ended up in New York.) Their presence in Britain facilitated a fruitful collaboration with the British scientists, certainly sped up the discovery process, and raised British neuropharmacology into glory. Progress in Germany came to a halt, and the British dominance of the field would last for two decades. Then, after the great pioneers had clearly won the war against the sparks, they missed the final triumph by leaving to others the problem of proving the presence of neurotransmitters in the brain.

The author was drawn to the history of the discovery of neurotransmitters while writing an earlier book on drugs and mental health (*1*). In that book he writes:

> In pursuing the biochemical approach to mental disorders an enormous amount has been learned, but it is questionable how much has been learned about mental illness. We do not really know if a biochemical imbalance is the cause of any mental disorder, and we do not know how even the hypothesized biochemical imbalances could produce the emotional, cognitive, and behavioral symptoms that characterize any mental disorder.

I suppose that many readers would like to know how Valenstein reconciles his strong

CREDIT: WELLCOME LIBRARY, LONDON

belief in the role of neurotransmitters for normal brain function with his doubts about a role for biochemical imbalances in mental disorders. For example, certain drugs are known to mimic mental disorders rather faithfully and at the same time to induce striking neurotransmitter imbalances. Is it far-fetched in such cases to propose some relation between biochemical and functional aberrations?

But my concern is somewhat tangential. *The War of the Soups and the Sparks* offers an excellent introduction to discoveries that provided the foundations for modern neuroscience. Valenstein's well-narrated account of one of the most fascinating chapters in the history of medical research can be strongly recommended.

Reference

1. E. Valenstein, *Blaming the Brain: The Truth About Drugs and Mental Health* (Free Press, New York, 1998).

10.1126/science.1120238

PHILOSOPHY OF SCIENCE

Genomic Meanings

Michael A. Goldman

The philosophy of molecular biology, Sahotra Sarkar contends, has lagged far behind that of evolutionary biology. *Molecular Models of Life*, a collection of his essays dated between 1988 and 2004, is intended to help shift philosophical thinking toward molecular biology "because—with the obvious exception of ecology—that is the biology that there is." Although that is a disturbing remark about the state of modern biology, it is happily inconsistent with the tenor of the rest of the book.

Sarkar holds a dual appointment in philosophy and integrative biology programs at the University of Texas at Austin. He has written widely, and his scholarly work over the years clearly bridges both fields. But I as a scientist found bewildering the forays into the nature of reductionism, and I imagine a philosopher would find it difficult to wade through arguments about interpretation of the Luria-Delbrück experiment (which demonstrated that in bacteria beneficial mutations arise randomly and not in response to selection).

A professional philosopher and biologist, Sarkar might be expected to see molecular biology as the outcome of an interplay among biochemistry, genetics, physics, and other fields. Evolutionary biology arose from natural history and geology, with later inputs from genetics and development. The historical separation between it and molecular biology may stem from the largely observational as opposed to experimental approaches of its roots. Now, however, we should appreciate the unifying concepts of different areas of biology. Thus, Sarkar's distinction between molecular biology and ecology is both too much of a separation and too little. If we recognize the two as discrete, then we must admit to a separate developmental biology, a physiology, a systems biology, a neurobiology, and even an evolutionary biology apart from ecology. We cannot imagine the perpetuation of the genes of higher metazoans in the absence of neural circuits or an environment with other interacting organisms, nor can we imagine an ecology entirely devoid of nucleic acids. We should see these fields as a continuum that might one day be understood as a whole, but which today is best studied in parts, using different methods and approaches.

Sarkar has been, over the years, a key thinker in the philosophy of molecular biology. One of his contentions is that the concept of information flow in biology is problematic. Sarkar repeatedly mentions the incompleteness or inadequacy of the central dogma of molecular biology. Although the idea of a genome as a program that spontaneously unfolds to produce a living organism is clearly too simplistic, that hardly renders the notion of information flow without value. A computer program, too, is totally dependent on its physical context in hardware and an operating system that can interpret it; its output is only as predictable as its input and can be rendered seemingly unpredictable by a temporary power surge or a scratch across a magnetic disk. We can recognize different inputs—including chance, environmental influences, and developmental context (e.g., maternal cytoplasmic effects)—in the interpretation of the genetic program, and we can even accept that some lines of that program (introns, intergenic regions) are of unknown function, without forgetting the program's key role in development.

Molecular Models of Life
Philosophical Papers on Molecular Biology
by Sahotra Sarkar
MIT Press, Cambridge, MA, 2005. 412 pp. $38, £24.95. ISBN 0-262-19512-7.

Perhaps because of his bleak outlook on the nature of information flow, Sarkar considers the Human Genome Project somewhat of a worthwhile failure. He notes how controversial the idea was even among geneticists and how tenuous the prospects for a full understanding of human biology and an incredible ability to cure diseases were. In retrospect, the project's early proponents may be forgiven their exaggerated promises. Few geneticists have ever proclaimed that day-to-day human behavior could be explained simply by gene interactions, and many have argued against attempts to connect behavioral traits and genetics. Nor, as Sarkar points out, did we imagine that there were so few genes, such a complex relation between genes and the protein forms they encode, and so much genetic material of unknown function. Nonetheless, we must understand that we can gain valuable insights from reading the human genome in all its variety.

By far the book's most intriguing chapter is its last, adapted from a forthcoming volume (*1*). Sarkar concisely captures most of the crucial history of the thinking in genetics and developmental biology (except for the influence of the eugenics movement) from about 1880 to the "completion" of the Human Genome Project. The essay seems an ideal capstone reading for my senior undergraduates and graduate students. In it, points made earlier in the book are refined to a pithy essence. Sarkar quotes Walter Gilbert's 1992 claim that the Human Genome Project will bring about "a change in our philosophical understanding of ourselves.... one will be able to pull a CD out of one's pocket and say, 'Here's a human being; it's me!'" Sarkar comments, "Today the claim seems laughable. None of the promises of Gilbert's radical genetic reductionism has been borne out. Proponents of the HGP promised enormous immediate medical benefits. Arguably, at least, there have not been any. Gilbert routinely promised the birth of a new theoretical biology. Instead, the emphasis now is on informatics...." On the upside, Sarkar notes that at "the very least, the HGP has killed the facile genetic reductionism of the heyday of developmental genetics." His dim view contrasts sharply with Robert Sinsheimer's recent proclamation that the project "succeeded even beyond our hopes" (*2*).

The book offers a collection of ideas and contributions rather than a much-needed synthesis. Sarkar attempts a summary in the first chapter, but there is otherwise little cohesiveness. Many ideas are repeated several times, some in practically the same words. At the dawn of systems biology, I am disappointed to find that field goes unmentioned in *Molecular Models of Life*. I would be hard-pressed to recommend the book—with the exception of the final chapter—to most readers of *Science* but equally at a loss to recommend a better one.

References

1. E. M. Neumann-Held, C. Rehmann-Sutter, Eds., *Genes in Development: Re-reading the Molecular Paradigm* (Duke Univ. Press, Durham, NC, 2005).
2. R. L. Sinsheimer, talk presented at the American Society of Human Genetics meeting, Salt Lake City, UT, 29 October 2005.

10.1126/science.1120191

The reviewer is in the Department of Biology, San Francisco State University, San Francisco, CA 94132–1722, USA. E-mail: goldman@sfsu.edu

EPIDEMIOLOGY

DNA Identifications After the 9/11 World Trade Center Attack

Leslie G. Biesecker,* Joan E. Bailey-Wilson, Jack Ballantyne, Howard Baum, Frederick R. Bieber, Charles Brenner, Bruce Budowle, John M. Butler, George Carmody, P. Michael Conneally, Barry Duceman, Arthur Eisenberg, Lisa Forman, Kenneth K. Kidd, Benoît Leclair, Steven Niezgoda, Thomas J. Parsons, Elizabeth Pugh, Robert Shaler, Stephen T. Sherry, Amanda Sozer, Anne Walsh

Before the September 11, 2001, World Trade Center (WTC) attack, the use of DNA profiling for victim identification in mass casualties (e.g., plane crashes) was typically limited to situations with fewer than 500 persons (*1*–*5*). Often, the condition of the remains allows rapid recovery of intact bodies. However, the number and condition of the remains in the WTC attack were unprecedented.

Enhanced online at www.sciencemag.org/cgi/content/full/310/5751/1122

After many deaths, especially in the aftermath of a mass fatality, there is an element of disbelief. Accepting the loss is a component of a complex grief process (*6*). Our motivation was to provide a tangible artifact of remains to survivors to facilitate coping and grief processes. Enabling family participation in some of the identification decisions was a critical component of the effort.

At the time of the attack, no infrastructure existed for rapid, effective victim identification in large-scale disasters (>1000 victims). Processes had to be scaled up to collect and analyze massive amounts of data in order to return identified remains to the families of almost 3000 victims. We summarize some challenges of the DNA-based component of the victim identification process, how these were met, and considerations for the future.

Some mass fatality identification projects begin with a list of victims (e.g., airline flight manifests listing passengers and crew). In contrast, the WTC mass fatality was initially "open" because the number of victims was unknown. Concerns about unreported or fraudulently reported victims made estimates difficult. The condition of the remains ranged from a few nearly complete bodies to multitudes of tiny fragments of charred bone, often difficult to distinguish from inorganic material. The fires affected the remains with temperatures exceeding 1000°C (*7*) that burned for more than 3 months. The towers' collapse fragmented and commingled victim remains and admixed building material. Many tissue fragments were retrieved months after the crashes, and bacterial and other processes further compromised the DNA. These factors made it difficult to isolate and genotype the DNA from the specimens.

Identification of human remains by DNA typing requires reference samples for comparison. These and other sources of information formed a deluge of material and data to be cataloged, archived, and analyzed. Preexisting sample collection and identification methods were insufficient for these needs.

Identification of WTC victim remains was the responsibility of the New York City Office of Chief Medical Examiner (OCME), which is one of the largest and most sophisticated in the country. Yet, its resources and scope of experience had to be expanded. The New York State Police Department (NYSP) was responsible for DNA analysis of reference samples. Several private DNA laboratories also tested samples, and software vendors helped to develop data analysis and compatibility tools. The NYSP and the OCME asked the National Institute of Justice (NIJ) to convene a group of scientific and medical experts [the Kinship and Data Analysis Panel (KADAP)] to advise them in the DNA identification effort. KADAP included experts in forensics; bioinformatics; and molecular, medical, statistical, and population genetics. The KADAP's charge was to assist the OCME in the development of procedures, standards of evidence, and processes related to the DNA identification effort. The final determination of a specific identification rested with the OCME (*8*).

DNA identification technologies. A battery of genetic identification markers, the Combined DNA Index System 13-locus short tandem repeat (STR) panel (CODIS) (*9*), was initially selected because it was established in forensic and legal systems and was compatible with forensic software packages. The first round of STR genotyping had a relatively low yield, because of DNA damage and other factors. Therefore, KADAP recommended several other approaches.

Because there are many more copies of mitochondrial DNA (mtDNA) than nuclear DNA, mtDNA analysis can be successful when DNA is limiting (*10*, *11*). Although mtDNA typing is generally labor-intensive, mtDNA typing with semiautomated analysis was provided for this project. Alone, mtDNA typing is insufficient to meet the threshold of identification and could only be used in conjunction with STR profiles. KADAP also recommended use of "mini" STR markers (*12*–*16*) that encompass the same CODIS STRs, but use shorter amplicons, which makes them more likely to be successful on fragmented DNA. Finally, technology for typing single-nucleotide polymorphisms (SNPs) was considered. Similar in concept to the use of mini-STRs, SNP typing can work with fragmented DNA because the amplicons are small.

Statistical criteria. About 5000 persons were initially assumed missing, so we used 1/5000 as the prior probability that a tissue fragment was from a particular missing person. This prior was lowered to 1/3000 as the victim list was refined. Direct DNA profile comparisons were used to test for identity

PLANNING FOR FUTURE DISASTERS

- Further research and development of forensic DNA typing systems is needed.
- Software must be able to integrate analytical, database, and workflow functions.
- Information technology infrastructure must be adequate to interconnect data-gathering, analysis, archiving, and reporting functions (*22*, *23*).
- A kit should be developed for mass-fatality reference sample and kinship data collection, as well as for family education and counseling.
- External prioritization requests should be minimized.
- Processes should be designed to test and to validate novel identification processes concurrent with their development.
- Criteria for determining end points should be designated early in the identification process (*24*).

*Authors were members of the World Trade Center Kinship and Data Analysis Panel. Affiliations are provided in Supporting Online Material. The opinions expressed here are those of the authors.

*Author for correspondence. E-mail: leslieb@helix.nih.gov

of tissue samples to reference samples from victims when these could be obtained. The CODIS marker set typically produces a random match probability (the probability of finding the same DNA profile in a randomly selected, unrelated individual) that is much less than 10^{-10} in most populations. This random match probability, when combined with the prior probability, yields the final match probability (*17*).

KADAP chose an initial minimum random match probability threshold (10^{-10}). This was stringent, but there was consensus that the approach would permit the OCME to make the easier identifications promptly and with high confidence. As the project progressed, fewer novel DNA profiles were generated, and the victim count decreased. Therefore, the required random match probability was reduced to 2.5×10^{-10} when DNA-based gender testing gave no results, to 5×10^{-9} for females, or 5×10^{-10} for males when gender markers were available (*18*). Details of these methods are described elsewhere (*19*). Although they represented a wide range of continental origins, many victims' backgrounds were unknown, so data on allele frequencies were not available for all groups. KADAP recommended that random-match probability be calculated using frequency data from each of four major population groups. The result that gave the most conservative random match probability was used (*20*).

Kinship analyses (comparison of a WTC remains DNA profile to those of biological relatives of the victims) were used when victim reference samples were not available or to confirm or increase confidence in direct comparisons. A kinship likelihood ratio was calculated for a victim sample by using family reference data, calculated jointly when multiple kin samples were available (*17*). This likelihood ratio compares the probabilities of observing a given DNA profile if a victim sample belonged to a particular missing individual (based on the stated genetic relationship of kin providing reference samples) to the chance of observing the profile if it was from an unrelated person. This likelihood ratio, when multiplied by the prior odds, yields the posterior odds (of kinship). To reach KADAP's goal of high confidence of correct identification, sufficient loci and family members were needed to reach a likelihood ratio of at least 3×10^6:1 (in favor of the stated genetic relationship).

Software issues. Matching thousands of victim sample genotypes (and partial genotypes) to next-of-kin and/or reference samples meant new software tools were needed. Data format incompatibilities and difficulties interconnecting the data set were major technical challenges. Contractors wrote custom "middleware" interfaces to unify several database and analytic functions. This provided a virtual tool linking many previously independent functions and freed the analysts from manually moving data among different platforms. Prioritizing samples was critical to maintaining high throughput. Work flow and tracking software modules were also developed. Protocols and software for evaluating and performing quality control of the software and analytic processes were designed and implemented (*21*).

The OCME recognized that its computers and data communication facilities were inadequate for this project. Data transfer between the NYSP and the OCME required new information technology infrastructure and support. A primary data repository allowing shared access to analysts outside OCME was set up on a secure server at the National Center for Biotechnology Information, in Bethesda, Maryland.

Because collection of personal reference and kinship samples was implemented rapidly, ~1/6 of the initial data had to be corrected or resampled. The KADAP developed new kinship and personal reference collection forms and components for standardized sample collection kits for future collections.

Summary of the effort and thoughts for the future. The OCME cataloged 19,913 putative victim tissue fragments from 2749 individuals reported missing. The fragment count increased to 20,120 because anthropological review identified commingled fragments (confirmed by DNA profiles). The DNA identification project generated more than 52,000 STR, 44,000 mtDNA, and 17,000 SNP profiles. As of September 11, 2005, about 850 of the 1594 victim identifications established for the 2749 WTC victims were based solely on DNA results. Most DNA identifications used standard CODIS genotypes. Although many CODIS genotypes originally failed, technical improvements leading to better DNA yields from damaged samples gave useful DNA profiles in 40% of the samples for which standard procedures failed. Modifications included a two-stage drilling process to isolate uncontaminated bone powder from the compromised specimens and modifications to the wash incubations, buffer concentrations, and elution times for the DNA isolation kit (*16*). Beyond standard CODIS STR typing technologies, more than 20% of the DNA identifications were made solely from mini-STRs. SNP analysis alone identified about 10 individuals with 10 more identifications made when SNP genotypes were used to supplement partial STR profiles. Additional identifications were made when mtDNA typing results were used to screen for potential matches, followed by DNA re-extraction and mini-STR retyping. No DNA-based identifications were accomplished by mtDNA analysis alone, as expected. The rate of new identifications has become negligible. The OCME and the KADAP believe that additional large-scale efforts are scientifically unwarranted at this time.

In looking toward the future, the KADAP panel recognized several major needs for improvements in technology and infrastructure (see table, p. 1122) (*22*). There is no doubt that improved preparedness and enhanced mass fatality forensic infrastructure would lead to more rapid and efficient identifications in the event of future mass disasters or terrorist attacks.

References and Notes

1. J. Ballantyne, *Nat. Genet.* **15**, 329 (1997).
2. B. Olaisen, M. Stenersen, B. Mevag, *Nat. Genet.* **15**, 402 (1997).
3. F. R. Bieber, in *DNA and the Criminal Justice System*, D. Lazer, Ed. (MIT Press, Cambridge, MA, 2004), pp. 23–62.
4. B. Leclair, C. J. Fregeau, K. L. Bowen, R. M. Fourney, *J. Forensic Sci.* **49**, 939 (2004).
5. A. Alonso *et al.*, *Croat. Med. J.* **46**, 540 (2005).
6. F. R. Bieber, *Harvard Med. Alumni Bull.* **76**, 34 (2002).
7. National Institute of Standards and Technology (http://wtc.nist.gov/NISTNCSTAR1-5F.pdf).
8. Z. M. Budimlija *et al.*, *Croat. Med. J.* **44**, 259 (2003).
9. B. Budowle *et al.*, in *Second European Symposium on Human Identification* (Promega Corporation, Madison, WI, 1998), pp. 73–88.
10. T. J. Parsons, M. D. Coble, *Croat. Med. J.* **42**, 304 (2001).
11. B. Budowle *et al.*, *Annu. Rev. Genomics Hum. Genet.* **4**, 119 (2003).
12. A. Hellmann, U. Rohleder, H. Schmitter, M. Wittig, *Int. J. Legal Med.* **114**, 269 (2001).
13. P. Wiegand, M. Kleiber, *Int. J. Legal Med.* **114**, 285 (2001).
14. K. Tsukada *et al.*, *Leg. Med.* (*Tokyo*) **4**, 239 (2002).
15. J. M. Butler, Y. Shen, B. R. McCord, *J. Forensic Sci.* **48**, 1054 (2003).
16. M. M. Holland *et al.*, *Croat. Med. J.* **44**, 264 (2003).
17. I. W. Evett, B. S. Weir, *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists* (Sinauer, Sunderland, MA, 1998).
18. About four times more males than females worked at the WTC, so the statistical threshold of a female profile (by the amelogenin marker) was higher than for a male. The reduction in stringency also meant if one fragment sample was matched to a reference sample by the criteria, a second fragment with a partial profile could also be matched if the random match profile probability of the two victim fragment samples was at least 1×10^{-8}.
19. C. H. Brenner, B. S. Weir, *Theor. Popul. Biol.* **63**, 173 (2003).
20. The population group reference DNA panel comprised 525 samples previously collected by the OCME; African American (n = 126), Caucasian (n = 123), Hispanic (n = 151), and Asian (n = 125).
21. National Center for BioTechnology (www.ncbi.nlm.nih.gov/IEB/Research/GVWG/OSIRIS/index.htm).
22. A "Lessons learned" document will be available through the National Institute of Justice: (www.ojp.usdoj.gov/nij/).
23. B. Budowle, F. R. Bieber, A. J. Eisenberg, *Leg. Med.* (*Tokyo*) **7**, 230 (2005).
24. KADAP attempted to determine an end point early in the process, but this could not be done because of unknown variables and the overwhelming nature of the attacks. The development of principles to facilitate such determinations would be useful.

25. We dedicate this to the memory of the victims of the WTC attack, to the families, and to everyone whose efforts made the identifications possible. We gratefully acknowledge Mark Dale for his advocacy and support.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1122/DC1

10.1126/science.1116608

CELL BIOLOGY

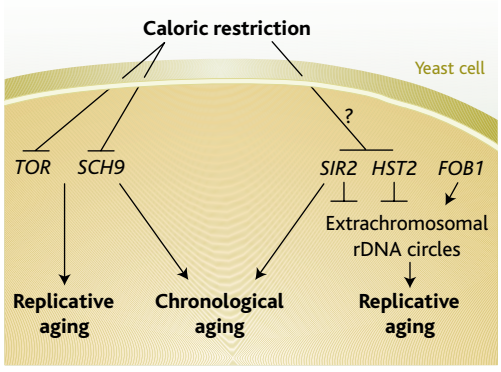
Twists in the Tale of the Aging Yeast

Jasper Rine

The study by Kaeberlein *et al*. on page 1193 of this issue (*1*), a recent *Science* paper by Lamming *et al*. (*2*), and a report by Fabrizio *et al.* (*3*) present the three latest chapters of the fascinating saga of how the life span of the budding yeast, *Saccahromyces cerevisiae*, is regulated (see the figure). To put this work into context, yeast mortality was a minor concern of humans until a study revealed that yeast have an ortholog of the human gene in which mutations cause Werner syndrome. This condition has characteristics that resemble premature aging. Mutations in the yeast version of this gene results in substantial shortening in one measure of yeast life span: the number of times a cell can divide, which is defined as its replicative life-span (*4*). This conserved feature of life span regulation in yeast and metazoans was thus of particular interest to all scientists, especially those of us past a certain age.

Subsequent work built upon this lead and found that *SIR2*, a gene originally famous for its role in nicotinamide adenine dinucleotide (NAD^+)–dependent deacetylation of histones in gene silencing, also promotes longevity of yeast by suppressing recombination in the repeated array of ribosomal DNA (rDNA) genes. This suppression blocks the accumulation of extrachromosomal circles of rDNA, whose abundance normally limits longevity and leads to the death of yeast mother cells. This result was fabulously interesting in the yeast field but lacked resonance in other organisms where there was no known link between aging and rDNA recombination.

Caloric restriction has long been recognized as the most common contributor to longevity in a range of organisms. Interest in yeast aging was reignited by the discovery that it too is extended by caloric restriction. The first paper to look at the involvement of *SIR2* in the link between caloric restriction and yeast aging (*5*) encountered some complexity: rDNA recombination in yeast is repressed by *SIR2* but is enhanced by *FOB1*, a gene necessary for a replication fork barrier between rDNA repeats. In *sir2* mutant cells with normal Fob1 function, extrachromosomal rDNA circles accumulate and limit longevity. Nonetheless, in a *fob1* mutant, caloric restriction was reported to extend life span in a *SIR2*-dependent way. Because caloric restriction would lead to more NAD^+ and less of its reduced form, NADH, this result suggested that Sir2 protein and its NAD^+ cofactor may be a universal mediator of the effect of caloric restriction on longevity. The excitement was all the more enjoyable because resveratol, an agonist of Sir2 enzymatic activity, is present in red wine, giving a new excuse for the pleasure of self-medication.



Effects of *SIR2* and *SCH9* on replicative and chronological life span. Caloric restriction operates independently of *SIR2* and its cousin *HST2* to promote replicative life span, as long as extrachromosomal rDNA circles are prevented by the action of *SIR2* and *HST2*. In contrast, *SIR2* prevents the extension of chronological life span by caloric restriction.

The preeminence of *SIR2* took a surprising hit when a study showed that extension of yeast life span by caloric restriction was *SIR2*-independent. Specifically, caloric restriction could, in fact, extend the life span of yeast lacking both *FOB1* and *SIR2*. The discrepancy was due to reliance in the earlier work on a uniquely odd yeast strain (*6*). The view in the field was that the high level of recombination in rDNA in *sir2* mutants created a potentially yeast-specific mechanism of aging that masked the ability of caloric restriction to extend life span. Only when rDNA recombination was vastly reduced in a *fob1 sir2* double mutant could caloric restriction extend life span. In essence, the study predicted a gene or pathway for controlling life span in yeast in response to caloric restriction that was both *SIR2*-independent and independent of recombination in the rDNA.

Just as we were beginning to suspect that all that red wine consumption was in vain, faith was restored in *SIR2* and in one of its molecular cousins (*2*). In this study, the authors used a petri-plate assay for the "recombinogenic state" of rDNA chromatin to screen the yeast genome for genes that, like *SIR2*, could extend life span through effects on rDNA. Remarkably, *HST2*, one of the four paralogous cousins of *SIR2* in the yeast genome, can repress rDNA recombination and extend yeast life span when it is overproduced. Although caloric restriction can still extend the life span of *hst2* mutants, just as it did for *sir2* mutants, it cannot extend the life span of a *hst2 sir2* double mutant. Thus, although *SIR2* could not explain all the effects of caloric restriction on aging, the combination of *SIR2* and its *HST2* cousin comes close to doing so.

However, as clear and interesting as this work is, there are two pesky complications. First, if caloric restriction has effects on yeast longevity independent of rDNA stability, they would likely have been missed by this study because of the rDNA bias in the screen used to uncover the role of *HST2*. Second, the ability of *fob1* to suppress the aging phenotype of *sir2* mutants was crucial to the argument for a caloric restriction effect on longevity independent of *SIR2*. Recall that the elevated recombination of rDNA and creation of extrachromosomal rDNA circles in yeast cells expressing *FOB1* creates a form of aging that masks the impact of caloric restriction. Alas, it appears that *fob1* mutants cannot suppress rDNA recombination in the combined absence of both *SIR2* and *HST2* (*2*). Hence, there could still be a caloric restriction effect on yeast aging, independent of both *SIR2* and *HST2*, that would be masked by the extrachromosomal rDNA circles resulting from the heightened recombination in the rDNA.

The work of Kaeberlein *et al*. (*1*) breaks through the historical bias that influenced key previous studies of yeast aging by screening a collection of mutant yeast, each with a single gene deletion, for the influence of genes on longevity. The elegance of this screen lies not in its genetic sophistication, but rather in the intersection of pragmatism and dedication to measure directly the replicative life span of multiple cells from hundreds of mutants. The

The author is in the Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA. E-mail: jrine@berkeley.edu

results so far are fascinating. Mutant forms of *TOR1* and *SCH9* (genes encoding two protein kinases involved in nutrient sensing), as well as mutations in other genes of these nutrient-sensing pathways, extend the life span of yeast. *Tor* and *Sch9* mutants also extend the life span of *Drosophila melanogaster* and *Caenorhabditis elegans*, encouraging extrapolation of these results more broadly. Indeed, *SCH9* is an ortholog of *AKT*, a gene that encodes a protein kinase in the insulin–insulin growth factor pathway, that figures prominently in life span studies of mice and worms. The increased life span of these *Tor1* and *Sch9* yeast mutants is *SIR2*-independent, but at present there are no experimental data on whether *HST2*, or even another *SIR2* cousin, might be necessary. On one hand, the contribution of rDNA recombination to aging seems to be yeast-specific, and hence so are the roles of *SIR2* and *HST2* in rDNA recombination. On the other hand, the impact of *TOR* and *SCH9/AKT* on aging appears to be conserved, and hence their *SIR2*-independent effects on aging in yeast may well be rDNA-independent. Owing to the difficulty of the assay, the work reported by Kaeberlein *et al*. surveys only ~10% of yeast genes for their contributions to longevity. Hence, further advances are virtually certain as this work progresses.

These studies on replicative life span ignore chronological life span, which is of equal interest given that most somatic cells are not replicating for the major portion of their life span. In the third new twist to this story, Fabrizio *et al*. (*6*) report that rather than promoting chronological life span, *SIR2* limits chronological life span. Moreover, in *sir2* mutants, caloric restriction is needed to extend chronological life span, providing another example of a *SIR2*-independent response to caloric restriction. As with the recent work on *SCH9* and *TOR1* (*1*), there are no experimental data on whether *HST2*, or even another *SIR2* cousin, might be also involved. Clearly, studies of chronological life span are also likely to benefit from an unbiased screen of the yeast knockout mutant collection. Because of the nature of the chronological aging assay, it may be possible to exploit the molecular bar codes unique to each mutant to screen all viable mutants in parallel.

As we have seen already in the roller coaster ride of yeast aging research, even these fine new additions are unlikely to be the last surprises in the story. Although the analysis of single and double mutants has proven useful, there is nothing quite as revealing as the phenotypes of the right triple mutant, unless of course it is the critical quadruple mutant, genotypes that may provide even more twists to this story.

References

1. M. Kaeberlein *et al*., *Science* **310**, 1193 (2005).
2. D. W. Lamming *et al*., *Science* **309**, 1861 (2005); published online 28 July 2005 (10.1126/science.1113611).
3. P. Fabrizio, *et al*. *Cell* **123**, 655 (2005).
4. D. A. Sinclair, K. Mills, L. Guarente, *Science* **277**, 1313 (1997).
5. S.-J. Lin, P.-A. Defossez, L. Guarente, *Science* **289**, 2126 (2005).
6. M. Kaeberlein, K. T. Kirkland, S. Fields, B. K. Kennedy, *PLOS Biol.* **2**, E296 (2004).

10.1126/science.1121310

GEOCHEMISTRY

A Stranger in Paradise

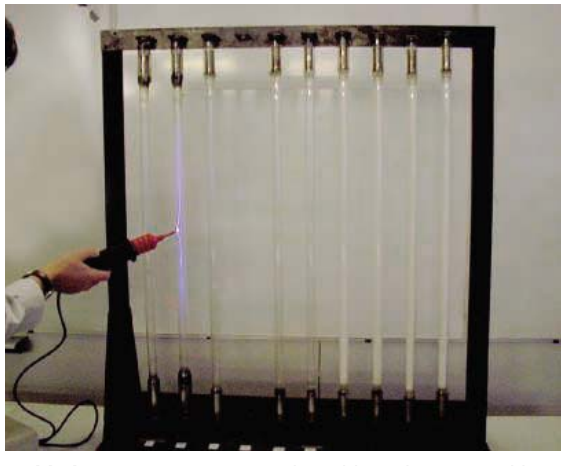
Paul F. McMillan

Why is the supposedly unreactive gas xenon depleted in the atmospheres of Earth and Mars, in contrast to its relatives neon, argon, and krypton? Could it possibly be reacting with silicate minerals in the crusts of these planets? On page 1174 of this issue, Sanloup *et al*. present high-pressure, high-temperature experimental results that suggest this could be the case (*1*). If so, another unexpected chapter in the already surprising story of this element might be beginning.

In 1783, Henry Cavendish was studying the formation of nitrous oxide, sparking mixtures of nitrogen and oxygen, when he made the observation that there was always a little chemically unreactive gas left over (*2*). However, he did nothing more on this, and his result lay dormant for more than a century. In 1894, Lord Rayleigh and Sir William Ramsay announced to the British Association the discovery of a new chemically unreactive element in the atmosphere that they named argon, "the lazy one." Ramsay went on to identify the other gaseous elements neon ("new"), krypton ("hidden"), and xenon ("alien" or "stranger"), so that a new column of elements must be added to Mendeleev's Periodic Table (*3*). He and Rayleigh were awarded the Chemistry and Physics Nobel prizes, respectively, in 1904 for their discoveries. Helium was identified first in the Sun and was later found emanating from terrestrial rocks. The chemical inertness of the "noble" gases was explained by Bohr's quantum theory: It was due to the special stability of their complete outer electron shells. The unreactivity of helium, neon, argon, krypton, and xenon became a fundamental principle of chemistry, and it gave rise to theories of ionic and covalent bonding that are still in use today (*4*).

That all changed in 1961, when Bartlett first noticed that the ionization energies of O_2 and xenon were nearly identical, and he then prepared the first xenon salt (originally formulated as $XePtF_6$) (*5*). Shortly afterward, XeF_4, XeF_2, $XeOF_4$, and XeO_3 were synthesized; today a whole range of compounds with xenon oxidation states ranging from +2 to +8 is known (*6*). Even compounds with xenon-carbon bonds have been prepared. The beginnings of analogous krypton chemistry have also been described, although no hints of true argon or neon chemistry have been detected to date. The noble gases are definitely not chemically inert species, however, if the reaction conditions are made right.

The strange chemical behavior of xenon extends into biochemistry and physiology, where no such reaction chemistry is expected to occur. Unexpectedly, xenon (and also krypton or argon, under hyperbaric conditions) is an excellent anesthetic (*7*). Similar



CREDIT: R. QUESADA/UNIVERSITY COLLEGE LONDON

Noble but not inert. Xenon glows blue when excited by a high-voltage discharge. The other tubes contain samples of He, Ne, Ar, and Kr. This display rack of rare gas elements is in the chemistry department at University College London, where they were first isolated and identified by Sir William Ramsay (whose work in collaboration with Lord Rayleigh at the Royal Institution led to the discovery of argon).

The author is in the Department of Chemistry and Materials Chemistry Centre, University College London, London WC1H 0AJ, UK, and the Davy-Faraday Research Laboratory, Royal Institution, London WBX 4BS, UK. E-mail: p.f.mcmillan@ucl.ac.uk

narcotic properties are recorded for chemically unreactive N_2 gas, giving rise to "l'ivresse des grandes profondeurs" (the "rapture of the deep"—the intoxication experienced by divers) (*8*). An early explanation for xenon anesthesia was given by Pauling, who suggested that clathrate hydrate structures encapsulating the rare gas atoms were formed near synapses, impeding interneuronal transmission (*9*). Recent results suggest a more interesting solution. The protein complexes that form transmembrane ion pumps associated with neurotransmitters contain hydrophobic regions. It is thought that neutral species such as xenon and N_2 might enter these regions, especially at high pressure, and interfere with the neuronal process to result in anesthesia and narcosis (*10*, *11*).

Highly oxidizing conditions are usually needed to activate xenon into true chemical reactivity and to form bonds with species such as oxygen. However, Sanloup *et al.* have used optical spectroscopy and synchrotron x-ray diffraction combined with chemical analysis to show that xenon can react with natural silicate materials, including SiO_2, to form xenon oxide species under the high-pressure, high-temperature conditions found in Earth's crust (*1*). This is an important result, because the noble gases form useful geochemical tracers. Formed by radioactive decay processes or encapsulated within deep Earth materials since the formation of the planet, the "inert" gaseous elements are thought to diffuse out from the mantle, core, and crust at well-defined rates. Xenon is particularly important in this regard. If it does undergo redox reactions and enter into chemical combination with silicates and other oxides, this could explain the apparent "xenon deficit" in the atmospheres of Earth and Mars, remarked upon by geochronologists and geophysicists (*12*). If this is true for xenon, then perhaps it also occurs for the radioactive rare gas, radon, that is formed by radioactive decay processes in crustal rocks.

The physical properties of xenon in deep Earth environments are as strange as its possible chemical behavior. Jephcoat (*13*) has shown that under lower mantle and core conditions, the melting point of xenon exceeds that of iron, as does its density. This means that if xenon did not react chemically with mantle or core materials, it would fall as "hail" toward the center of Earth, through the molten outer core. However, the results of Sanloup *et al.* suggest that it can also react with silicates, oxidizing them to metallic alloys or replacing silicon in mineral structures. There might be new geochemical partitioning equilibria to be considered within the deep crust, mantle, and core, involving xenon physics and chemistry.

It is of interest that Sanloup *et al*. carried out their experiments in platinum capsules, because reactions involving platinum were how Bartlett initiated his first xenon chemistry experiments (*5*). Perhaps the capsules are not completely innocent in the high-pressure and high-temperature experiments, because they might act as reaction sites for xenon in addition to providing the host for platinum-silicon alloys formed during the presumed silicate reduction reactions. However, even if that is the case, it does not rule out the potential importance of metal-silicate reactions involving xenon within Earth. These results could presage a new area in xenon solid-state chemistry under high-pressure conditions, which might be extended to other noble gases that have not yet been chemically awakened, if the conditions are made right.

References and Notes

1. C. Sanloup *et al.*, *Science* **310**, 1174 (2005).
2. G. Wilson, *The Life of the Honourable Henry Cavendish* (Cavendish Society, London, 1851).
3. W. Ramsay, *The Gases of the Atmosphere, the History of Their Discovery* (Macmillan, London, 1915).
4. G. N. Lewis, *J. Am. Chem. Soc.* **38**, 762 (1916).
5. N. Bartlett, *Proc. Chem. Soc.* **1962**, 218 (June 1962).
6. N. N. Greenwood, A. Earnshaw, *Chemistry of the Elements* (Pergamon, Oxford, 1984).
7. N. P. Franks, R. Dickinson, S. L. de Sousa, A. C. Hall, W. R. Lieb, *Nature* **396**, 324 (1998).
8. J. Cousteau, *The Silent World* (Reprint Society, London, 1953).
9. L. Pauling, *Science* **134**, 15 (1961).
10. P. Bennett, D. Elliott, *The Physiology and Medicine of Diving* (Saunders, New York, ed. 5, 2003).
11. N. P. Franks, W. R. Lieb, *Nature* **300**, 487 (1982).
12. E. Anders, T. Owen, *Science* **198**, 453 (1977).
13. A. P. Jephcoat, *Nature* **393**, 355 (1998).
14. The author is supported by a Wolfson Royal Society Research Merit Award Fellowship.

10.1126/science.1121022

PALEONTOLOGY

Dinosaurs Dined on Grass

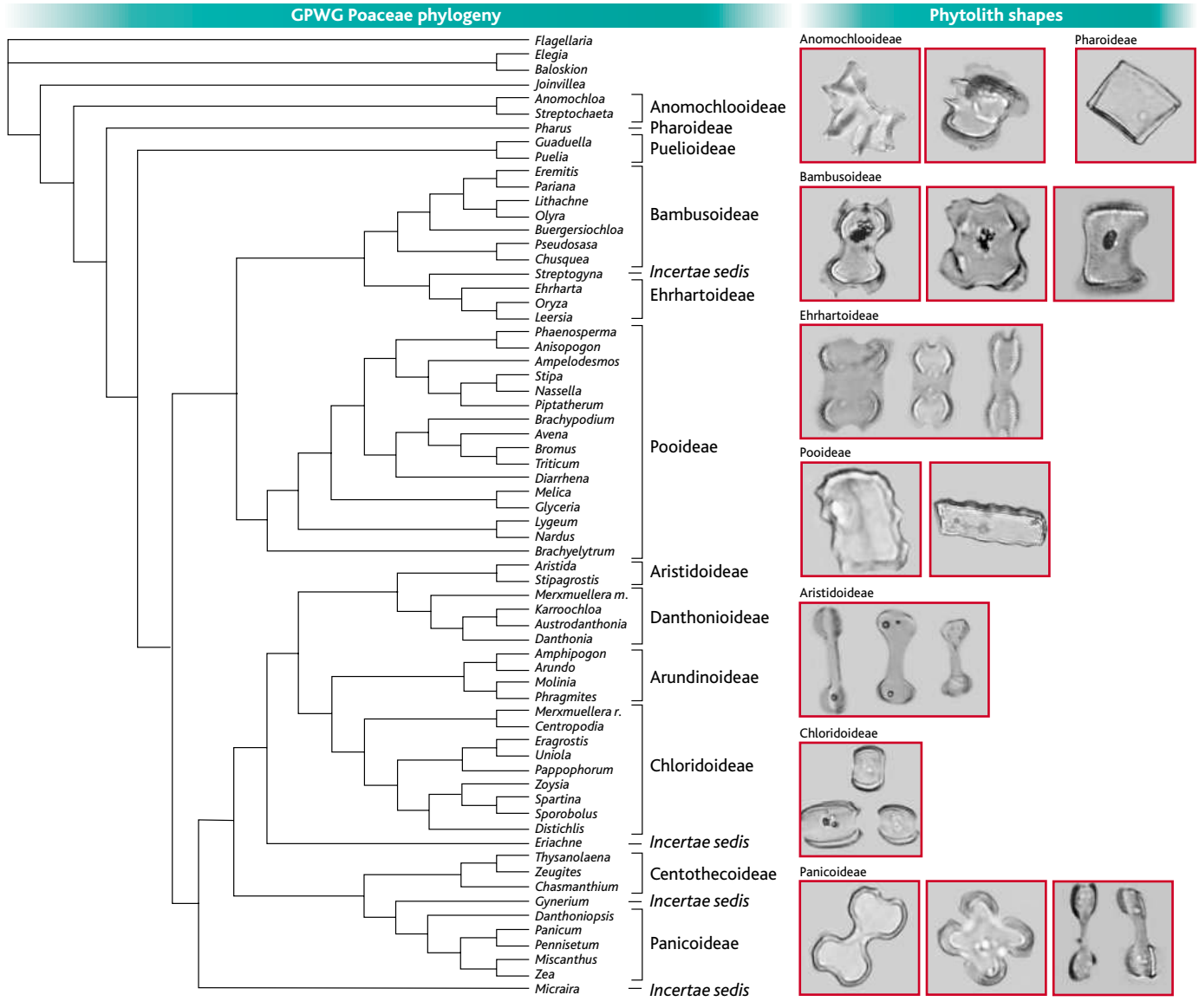
Dolores R. Piperno and Hans-Dieter Sues

Grasses (family Poaceae or Gramineae), with about 10,000 extant species, are among the largest and most ecologically dominant families of flowering plants, and today provide staple foods for much of humankind. Dinosaurs, the dominant megaherbivores during most of the Mesozoic Era (65 to 251 million years ago), are similarly one of the largest and best known groups of organisms. However, the possible coevolution of grasses and dinosaurs has never been studied. Now, Prasad *et al.* (*1*) report on page 1177 of this issue their analysis of phytoliths—microscopic pieces of silica formed in plant cells—in coprolites that the authors attribute to titanosaurid sauropods that lived in central India about 65 to 71 million years ago. Their data indicate that those dinosaurs ate grasses.

Part of the difficulty in studying the question of dinosaur-grass coevolution results from the poor quality of the fossil record for early grasses. The earliest unequivocal grass fossils date to the Paleocene-Eocene boundary, about 56 million years ago (*2*, *3*), well after the demise of nonavian dinosaurs at the end of the Cretaceous Period. Pollen and macrofossils of Poaceae are uncommon in sedimentary strata until the middle Miocene, about 11 to 16 million years ago, when the family is thought to have undergone considerable evolutionary diversification and ecological expansion (*2*). Thus, dioramas in museums have long depicted dinosaurs as grazing on conifers, cycads, and ferns in landscapes without grasses. The work of Prasad *et al.* (*1*) is the first unambiguous evidence that the Poaceae originated and had already diversified during the Cretaceous. The research shows that phytoliths, which have become a major topic of study in Quaternary research over the last 20 years (*4*–*8*), can provide a formidable means for reconstructing vegetation and animal diets for much earlier time periods when early angiosperms were diversifying. These remarkable results will force reconsideration of many long-standing assumptions about grass evolution, dinosaurian ecology, and early plant-herbivore interactions.

Scientists have long known that grasses make distinctive kinds of phytoliths in the epidermis of their leaves and leaflike coverings that surround their flowers (*9*). More recent work has examined in greater detail phytolith characteristics from a large set of grasses comprising taxa representing the entire range of diversification within the family, showing that discriminations at the subfamily, tribe, and genus levels are often possible (*1*, *4*–*8*, *10*). In addition, publication of a well-resolved consensus phylogeny of the Poaceae by the Grass Phylogeny Working Group (GPWG) (*11*) considerably advances our overall understanding of the evolutionary history of grasses and leads to improved interpretations of the early grass fossil record. For example, by mapping the phytolith characters that discriminate clades and subfamilies of extant taxa onto this phylogenetic tree, we can infer how phytolith morphology changed at

The authors are at the National Museum of Natural History, Washington, DC 20560, USA. D. R. Piperno is also at the Smithsonian Tropical Research Institute, Balboa, Panama. E-mail: pipernod@si.edu



Grass lineage. (**Left**) Phylogeny for grasses from GPWG (*11*). (**Right**) Examples of phytoliths from basalmost and later-diverging families of grasses, showing that the earliest grasses probably contributed phytoliths unlike those in the BEP clade. Phytoliths typical of the Aristidoideae, Panicoideae and Chloridoideae (PACCAD clade) are also shown. Phytolith images are from the work of the authors (*4, 14*).

the origin of major clades and lineages (see the figure).

The most primitive grasses belong to the family Anomochlooideae, represented by two extant genera from tropical America, *Streptochaeta* and *Anomochloa*. They make distinctive, oddly shaped kinds of phytoliths not found in other extant grasses. The Pharoideae, the next group to diverge, make another type of unique phytolith. A notable point is that bilobate, cross-shaped, and conventional saddle-shaped phytoliths, the types long used by researchers to define grass silica deposition, appear to be absent from basal grasses (*1*, *10*). They apparently made their first appearance in the later-diverging Puelioideae (producers of saddle-shaped phytoliths) and BEP (Bambusoideae/Ehrhartoideae/Pooideae) taxa. The Bambusoideae and Ehrhartoideae (bamboos, rice relatives) in particular possess many diverse bilobate, saddle- and cross-shaped phytoliths, and some are diagnostic of each subfamily (see the figure). These also differ substantially from the more simple, mirror-image kinds of phytoliths well described in the large PACCAD (e.g., Panicoideae and Chloridoideae) clade of grasses (*1*, *4*, *5*, *8*–*10*).

In the dinosaur coprolites studied by Prasad *et al*., phytoliths indisputably derived from grasses are common. It is noteworthy that phytoliths diagnostic of the most basal grasses (those found in the modern genera *Anomochloa*, *Streptochaeta*, and *Pharus*) are absent, whereas types produced today in the Puelioideae, Ehrhartoideae, and Bambusoideae were frequently encountered. Moreover, the "vertical" bilobate and cross-shaped phytoliths observed by Prasad *et al.* [see figure 2, a and b, of (*1*)] are confined to a few extant genera of the ehrhartoidean tribe Oryzeae, and other reported phytoliths are probably from the Pooideae. Therefore, the conclusion that a considerable amount of pre-Tertiary diversification occurred among the Poaceae, leading to a much earlier emergence and radiation of the major BEP clade than had been previously thought on the basis of fossil and molecular clock data, is well supported. PACCAD-clade grasses may also have been present, but this finding is more ambiguous at the present time because of the possibility of morphological overlap with basal Pooideae.

These results also have considerable importance for investigations of ancient plant-herbivore interactions, a major field of study because coevolutionary relationships that arise between plants and their herbivores have

major, diverse effects on each group (*12*). The documentation by Prasad *et al*. of a range of poacean taxa in the Late Cretaceous of India makes the possibility real that titanosaurid sauropods were not the only grass eaters of the era, and that coevolutionary interactions between grasses and diverse vertebrate herbivores may have greater antiquity than previously believed. For example, the enigmatic gondwanatherian mammals with their high-crowned (hypsodont) cheek teeth could have eaten grasses (*13*). It has often been argued that the intense consumption of vegetation by herd-forming herbivorous dinosaurs led to the diversification of angiosperms during the Cretaceous (*12*). Prasad *et al*. identified the silicified remains, including trichome phytoliths, of a variety of nongrass angiosperms in the coprolites, providing direct evidence that the dinosaurs were generalist herbivores. Phytoliths are common in an array of extant basal angiosperms, monocotyledons, and eudicotyledons in addition to grasses and are among the few substances capable of inducing morphological changes to animal mouthparts (*14*). It is believed that they constitute an important type of mechanical plant defense against both insect and vertebrate herbivory (*14*). By 65 million years ago, therefore, angiosperms may have experienced considerable herbivore pressure such that some had evolved simple, inexpensive mechanical defenses that involved impregnating their structures with silica. The new data provided by Prasad *et al.* are certain to help resolve these and other important issues in Mesozoic terrestrial ecology.

References

1. V. Prasad, C. A. E. Strömberg, H. Alimohammadian, A. Sahni, *Science* **310**, 1177 (2005).
2. B. F. Jacobs, J. D. Kingston, L. L. Jacobs, *Ann. Mo. Bot. Gard.* **86**, 590 (1999).
3. E. A. Kellogg, *Plant Physiol.* **125**, 1198 (2001).
4. D. R. Piperno, *Phytolith Analysis: An Archaeological and Geological Perspective* (Academic Press, San Diego, CA, 1988).
5. G. G. Fredlund, L. T. Tieszen, *J. Biogeogr.* **21**, 321 (1994).
6. G. G. Fredlund, L. T. Tieszen, *Quat. Res.* **47**, 206 (1997).
7. M. Blinnikov, A. Busacca, C. Whitlock, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **177**, 77 (2002).
8. D. M. Pearsall, K. Chandler-Ezell, A. Chandler-Ezell, *J. Archaeol. Sci.* **30**, 611 (2003).
9. C. R. Metcalfe, *Anatomy of the Monocotyledons. I. Gramineae* (Oxford Univ. Press, London, 1960).
10. D. R. Piperno, D. M. Pearsall, *Smithson. Contrib. Bot.* **85**, 1 (1998).
11. Grass Phylogeny Working Group, *Ann. Mo. Bot. Gard.* **88**, 373 (2001).
12. H.-D. Sues, Ed., *Evolution of Herbivory in Terrestrial Vertebrates* (Cambridge Univ. Press, Cambridge, 2000).
13. R. Pascual *et al.*, *J. Vertebr. Paleontol.* **19**, 373 (1999).
14. D. R. Piperno, *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists* (AltaMira, Lanham, MD, in press).

10.1126/science.1121020

GENETICS

Two Genes Link Two Distinct Psychoses

Akira Sawa and Solomon H. Snyder

During the past decade, the tools of molecular genetics have begun to bear fruit in searches for selective genes involved in the major psychotic illnesses, schizophrenia and affective disorder. The most direct evidence involves the gene *DISC1* (*disrupted in schizophrenia 1*) (*1*). On page 1187 of this issue (*2*), Millar *et al*., who pioneered the discovery of *DISC1* (*3*), now report a chromosomal translocation in schizophrenia involving the gene encoding phosphodiesterase 4B, *PDE4B*. DISC1 binds PDE4B1, but an increase in cellular cyclic adenosine monophosphate (cAMP) dissociates the proteins and activates the phosphosdiesterase. This discovery affords a molecular explanation of cognitive and affective dysfunction and may clarify the relationship between schizophrenia and affective illness, thus potentially leading to new therapeutic strategies.

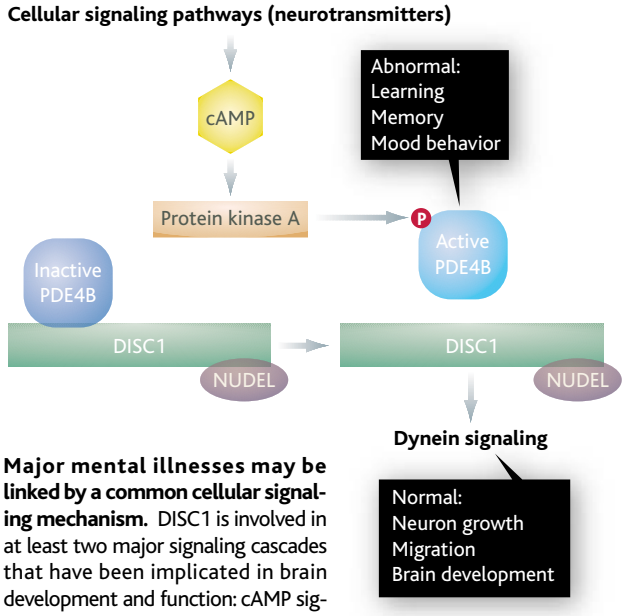
Genetic linkage and association studies have implicated several candidate genes in schizophrenia, including those encoding neuregulin 1, dysbindin, and regulator of G protein signaling 4 (*4*). In contrast to these suggestive findings, *DISC1* is the first gene whose chromosomal aberrations clearly segregate with psychotic disturbance. This includes both schizophrenia and affective disorder, with direct involvement in at least two distinct pedigrees (*5*, *6*). Furthermore, linkage and association studies establish *DISC1* as a candidate susceptibility gene in large populations of patients with schizophrenia or affective disorder (*4*). The intriguing relationship of *DISC1* to both of these disorders, long thought to be distinct, has been strengthened by a link to chromosome 1q42, the region of *DISC1*, for schizoaffective disorder. As the name implies, this mental disorder shares the characteristic of both cognitive and mood-related illnesses (*7*).

How *DISC1* influences mental function is beginning to be clarified. In schizophrenic patients, *DISC1* variants are linked to specific neurocognitive impairments (*8*–*11*). A single-nucleotide polymorphism in *DISC1* that leads to an amino acid change ($Ser^{704} \rightarrow Cys$) is associated with schizophrenia and correlates with variations in hippocampal size and function during cognitive tasks in normal subjects (*8*) as well as cognitive variations in aged normal subjects (*11*).

Cellular functions of DISC1 have been revealed through the identification of various proteins that interact with it: microtubule-centrosome–associated proteins such as NUDEL (see the first figure); actin-related proteins such as FEZ-1; postsynaptic density–related proteins such as citron; and nuclear proteins such as activating transcription factor 4 (*12*–*14*). DISC1 is critical for maintaining a complex containing NUDEL and the microtubule-associated motor protein dynein at the centrosome. Mutations in *DISC1*, such as those that produce truncated forms of the protein, prevent dynein-centrosome interaction in some schizophrenic patients (*15*). In addition, reduced expression of DISC1 in mice disturbs proper neuronal migration and arborization of dendritic neuronal processes in the developing cerebral cortex (*15*). These findings have suggested that localization of dynein to centrosomes through DISC1 is critical for dynein signaling that regulates the growth of neuronal processes and development of the cerebral cortex. The DISC1 mutation found in the Scottish pedigree could lead to a truncation of DISC1 at its carboxyl terminus and/or degradation of the protein. Failure to detect the truncated DISC1 in lymphoblasts of a few schizophrenics from the Scottish pedigree may also be attributed to variations in DISC1 protein isoforms (see the second figure). Such isoforms could exhibit differences in their metabolism in the brain and lymphoblasts as well as in the developing and adult brain (*1*, *16*). Loss of DISC1 and/or actions of the truncated mutant DISC1 can cause dysfunctions that would fit with substantial evidence that schizophrenia is a disorder of neural development (*17*–*19*).

Millar *et al.* (*2*) now report a patient with a chromosomal translocation involving the gene encoding PDE4 that leads to a 50% reduction in expression of PDE4B1, one subtype of PDE4B. They detect binding of PDE4B1 with DISC1 (see the second fig-

The authors are in the Departments of Psychiatry and Neuroscience and the Program in Cellular Molecular Medicine, and S. H. Snyder is also in the Department of Pharmacology, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA. E-mail: asawa1@jhmi.edu; ssnyder@jhmi.edu



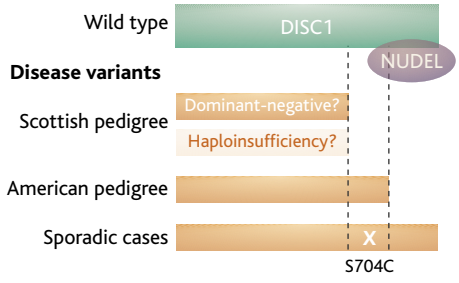
Major mental illnesses may be linked by a common cellular signaling mechanism. DISC1 is involved in at least two major signaling cascades that have been implicated in brain development and function: cAMP signaling and dynein-NUDEL signaling. Increases in cellular cAMP and protein kinase A activity disrupt DISC1-PDE4B interaction. This augments phosphodiesterase activity and increases the dynein signaling pathway, which have been linked to alterations in brain development, cognition, and mood behavior.

ure). Moreover, drug-induced augmentation of intracellular cAMP interferes with the binding of PDE4B1 to DISC1. This dissociation is related to phosphorylation of PDE4B by cAMP-dependent protein kinase (protein kinase A), because a selective inhibitor of protein kinase A reverses dissociation of the DISC1-PDE4B1 complex. Blocking the binding of DISC1 to PDE4B1 increases phosphodiesterase activity. Thus, DISC1 appears to bind primarily to the dephosphorylated form of the PDE4B1 that has diminished catalytic activity. Signaling systems that increase cellular levels of cAMP, such as those triggered by neurotransmitters, could consequently lead to phosphorylation of PDE4B and increased phosphodiesterase activity. We do not yet know the influence of DISC1-PDE4B1 interaction on dynein signaling.

The discovery of PDE4B as a key factor in schizophrenia is tantalizing because the enzyme has been extensively implicated in affective and cognitive function. Cognitive impairment has been observed in a mutant strain of the fruit fly *Drosophila melanogaster* that expresses nonfunctional PDE4 (*20*). Moreover, the antidepressant drug rolipram is a PDE4 inhibitor (*21*). The relationship of PDE4 to mood would fit best with a role in affective disorder, but the single patient described by Millar *et al.* was diagnosed as schizophrenic and a second patient has an unspecified psychotic disturbance. It is possible that, as with DISC1, when more patients with PDE4 disturbances appear, a substantial number will manifest affective disorder.

How might mutant DISC1 and PDE4B mediate the symptoms of psychosis? Insofar as schizophrenia is a developmental disorder (*17*–*19*) and *DISC1* modulates neural development (*15*), *DISC1* disruption might explain the developmental brain abnormalities of schizophrenics (*1*, *17*). Disturbances of mood could be ascribed to *PDE4B*. Most fascinating are the potential influences on psychotic behavior caused by interactions between the two proteins: Because PDE4B1 is inactivated when bound to DISC1 but becomes active when dissociated from DISC1, decreased expression of DISC1 in patients should elicit increased phosphodiesterase activity. The DISC1-PDE4B link may help elucidate normal as well as diseased brain function because of the dynamic interaction between the two proteins. Many neurotransmitters affect cAMP directly or indirectly. Signals that increase the amount of intracellular cAMP would activate PDE4B, providing a negative feedback to then lower the elevated cAMP level. The converse would take place for signals that initially decrease cAMP.

Might therapeutic agents be developed on the basis of the DISC1-PDE4B1 interaction? Assuming that psychotic patients manifest a lowered level and function of DISC1, pathophysiology would be determined by increased phosphodiesterase activity. Hence, one might seek agents that facilitate the binding of the two proteins. Such agents should have the same impact as phosphodiesterase inhibitors and thus could be useful in treating depression. In contrast, abundant literature suggests that increasing cAMP-protein kinase A signaling may enhance memory (*22*) so that drugs blocking DISC1-PDE4B1 binding would be useful cognitive stimulants. But recent evidence suggests that cerebrocortical components of cognition are facilitated by decreased cAMP, in contrast to hippocampal memory that is improved with heightened cAMP–function (*23*). However tantalizing, the prediction of drug effects is still highly speculative.



Familial DISC1 mutations associated with psychosis. Thus far, two such familial DISC1 mutations have been reported in Scottish and American pedigrees. A nonsynonymous polymorphism ($Ser^{704} \rightarrow Cys$) in DISC1 is also implicated in major mental illness and cognitive dysfunction.

The intersection of DISC1 and PDE4 in schizophrenia may reflect an increasingly prominent paradigm in neuropsychiatric illness. Neurotransmitter signaling had been a principal focus of speculation regarding pathophysiology with abnormalities in dopamine and glutamate signaling highlighted in schizophrenia, serotonin in depression, acetylcholine in Alzheimer's disease, and γ-aminobutyric acid in Huntington's disease. Molecular investigations subsequently emphasized the role of cytoskeletal, scaffolding, and aggregating proteins such as huntingtin in Huntington's disease, amyloid precursor proteins in Alzheimer's disease, and DISC1 in schizophrenia. Now investigators perceive links such as amyloid β peptide aggregates altering the disposition of choline acetyltransferase, the acetylcholine-forming enzyme. The ability of DISC1 to alter phosphodiesterase activity—and hence to alter the synaptic influences of cAMP—can reconcile developmental disturbances in brain structure with aberrations in neurotransmitter-mediated information processing.

References

1. K. Ishizuka, M. Paek, A. Kamiya, A. Sawa, *Biol. Psychiatry*, in press.
2. J. K. Millar *et al.*, *Science* **310**, 1187 (2005).
3. J. K. Millar *et al.*, *Hum. Mol. Genet.* **9**, 1415 (2000).
4. P. J. Harrison, D. R. Weinberger, *Mol. Psychiatry* **10**, 40 (2005).
5. D. H. Blackwood *et al.*, *Am. J. Hum. Genet.* **69**, 428 (2001).
6. N. A. Sachs *et al.*, *Mol. Psychiatry* **10**, 758 (2005).
7. M. L. Hamshere *et al.*, *Arch. Gen. Psychiatry* **62**, 1081 (2005).
8. J. H. Callicott *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8627 (2005).
9. W. Hennah *et al.*, *Mol. Psychiatry* 10.1038/sj.mp4001731.
10. K. E. Burdick *et al.*, *Neuroreport* **16**, 1399 (2005).
11. P. A. Thomson *et al.*, *Neurosci. Lett.* **389**, 41 (2005).
12. Y. Ozeki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 289 (2003).
13. K. Miyoshi *et al.*, *Mol. Psychiatry* **8**, 685 (2003).
14. J. A. Morris, G. Kandpal, L. Ma, C. P. Austin, *Hum. Mol. Genet.* **12**, 1591 (2003).
15. A. Kamiya *et al.*, *Nat. Cell Biol.* in press.
16. N. Sawamura, T. Sawamura-Yamamoto, Y. Ozeki, C. A. Ross, A. Sawa, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 1187 (2005).
17. A. Sawa, S. H. Snyder, *Science* **296**, 692 (2002).
18. D. R. Weinberger, *Arch. Gen. Psychiatry* **44**, 660 (1987).
19. D. A. Lewis, P. Levitt, *Annu. Rev. Neurosci.* **25**, 409 (2002).
20. R. L. Davis, J. Cherry, B. Dauwalder, P. L. Han, E. Skoulakis, *Mol. Cell. Biochem.* **149**, 271 (1995).
21. J. Zhu, E. Mix, B. Winblad, *CNS Drug Rev.* **7**, 387 (2001).
22. M. G. Baxter, *Neuron* **40**, 669 (2003).
23. B. P. Ramos *et al.*, *Neuron* **40**, 835 (2003).

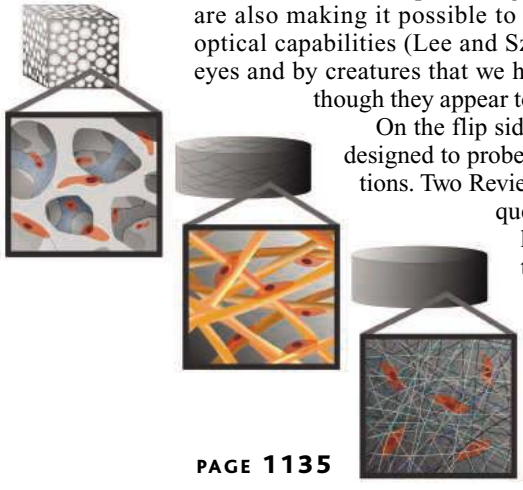
10.1126/science.1121114

INTRODUCTION

Design for Living

When a child sees a bird flying past or the fluttering wings of a butterfly, does it inspire thoughts about how to build airplanes? Or does it simply convey the idea that flight is possible? We are immersed in the natural world, so it is not surprising that it inspires the design of engineered structures, or that we would like to probe this world further to learn all its secrets.

The four Reviews in this issue highlight this two-sided relationship as it applies to the development of new materials. From nature we have learned how soft brittle materials like chalk are made tougher through composite structures. Some of these same design principles have been applied to the much wider range of building blocks available to the engineer (Mayer, p. 1144). Advances in materials processing, particularly in the area of polymers, are also making it possible to fabricate systems with advanced optical capabilities (Lee and Szema, p. 1149), inspired by living eyes and by creatures that we have recently learned can see even though they appear to lack eyes.



PAGE 1135

On the flip side, new material systems have been designed to probe and manipulate biological interactions. Two Reviews examine highly complementary questions. The first summarizes what has been learned about the interactions of cells with surfaces that possess features at different size scales (Stevens and George, p. 1135). The second explores how cells sense the stiffness and strength of underlying substrates (Discher *et al.*, p. 1139). From both we see how new materials are being used to probe cell cycles and interactions and to coax cells to grow in desired ways.

The drive toward medical applications is helping push research at the biology/materials interface. On the small scale, nanotechnology is being explored to find new methods for cancer detection and treatment (Service, p. 1132). A Science of Aging Knowledge Environment (SAGE KE) feature story by Davenport explores the progress and problems of engineering tissues in the lab. This endeavor could revolutionize medicine, potentially reversing illnesses such as diabetes, heart disease, and liver failure.

As research at the interface between materials and biology increasingly overlaps, we look forward to seeing how each continues to inspire developments in the other.

–MARC LAVINE, VALDA VINSON, ROBERT COONTZ

CONTENTS

See also related Brevia on p. 1151; SAGE KE material on p. 1083 or at www.sciencemag.or/sciext/materials/

CREDIT: M. STEVENS *ET AL.*

Science

NEWS

Nanotechnology Takes Aim at Cancer

The science of extremely small materials is poised to revolutionize cancer diagnostics, imaging, and treatment and could finally usher in the long-awaited era of personalized medicine

If there is a case to be made for personalized medicine, cancer is it. Every year, nearly 1.4 million Americans are diagnosed with the disease; another 600,000 die from it. Yet, although cancer is often portrayed as a monolithic illness, it is anything but. There are more than 200 types of cancers, each with many variants. Some are aggressive, some docile; some are easily treated, others are almost always fatal. Diagnosing, treating, and tracking the progress of therapy for each type of cancer has long been a dream among oncologists, and one that has grown closer thanks to parallel revolutions in genomics, proteomics, and cell biology. Now a new revolution in nanotechnology is pushing personalized cancer treatment closer than ever before.

Nanotechnology's ability to shape matter on the scale of molecules is opening the door to a new generation of diagnostics, imaging agents, and drugs for detecting and treating cancer at its earliest stages. But perhaps more important, it is enabling researchers to combine advances, creating nanosized particles that contain drugs designed to kill tumors, targeting compounds designed to home in on malignancies, and imaging agents designed to light up even the earliest stage cancers. "The future of oncology—and the opportunity to eliminate the suffering and death due to cancer—will hinge on our ability to confront cancer at its molecular level," says Andrew von Eschenbach, former director of the U.S. National Cancer Institute (NCI) in Bethesda, Maryland. Unlike previous "revolutions" in the "war" on cancer that raised hopes, nanotechnology "is not just one more tool, it's an entire field and will pervade everything in medicine," says Mauro Ferrari, a cancer nanotechnology expert at Ohio State University in Columbus.

These promises have already set off a burgeoning effort to marry nanotechnology with oncology. Most notably, in 2004, NCI launched a $144 million cancer nanotechnology initiative. As the foundation of this effort, last month NCI announced $26.3 million for the first year of funding for seven centers of cancer nanotechnology excellence designed to foster interdisciplinary work among chemists, materials scientists, and biologists. Europe and Japan are also investing heavily in nano approaches to fighting cancer, although nanotechnology funding agencies there don't break out specific programs for cancer. "It's fair to say [Europe and Japan] are putting in complementary amounts of money to the U.S. NCI," says Ruth Duncan, a nanomedicine expert at the Welsh School of Pharmacy in Cardiff, Wales. Companies are also getting in on the act: More than a half-dozen nanoparticle-based imaging agents and therapeutics are either on the market, in clinical trials, or awaiting clinical trials (see table, p. 1134).

"The science in this area is exploding."

The cancer community really gets this now."

—Gregory Downing, NCI

"The science in this area is exploding," says Gregory Downing, who heads NCI's Office of Technology and Industrial Relations. "The cancer community really gets this now." Thomas Kipps, a cancer biologist at the University of California, San Diego, agrees. "I think there is tremendous potential here," he says. "I hope it doesn't just turn out to be hype. But I don't think it will."

A softer touch

Cancer treatment has had more than its share of hype over the years. Yet despite progress in understanding cancer, its diagnosis and treatment have remained essentially unchanged for decades, and death rates from the disease are about what they were in 1950. "If you look at the everyday treatment of cancer, it's just like it was 30 years ago with just a couple of exceptions," says Michael Phelps, a cancer imaging expert at the University of California, Los Angeles. Chemotherapy, radiation, and surgery—the big three of treatments—all wreak havoc on healthy cells and tissues as well as cancerous ones. And the only way to tell whether they have worked is to wait to see whether the cancer reappears.

Nanotechnologists hope to break the log-jam by giving oncologists new tools for tracking and targeting cell surface receptors and other molecules specific to cancer cells. This push toward personalized medicine has been under way for years. For example, the cancer drug Herceptin, which homes in on a receptor called Her-2 that is overexpressed in certain cancer cells, is given only to patients whose diagnostic tests show they carry Her-2 positive cells. Nanotechnologists hope to extend that approach to numerous diagnostics, imaging agents, and medicines. "Cancer can benefit from nanotechnology in essentially every sector of the cancer enterprise," Ferrari says.

Raising red flags

Advances in diagnostics are already well under way in laboratories around the globe. In the October issue of *Nature Biotechnology*, for example, researchers led by Charles Lieber of Harvard University described using arrays of silicon-based nanowire devices (see figure, above) to electrically detect minute levels of marker proteins overexpressed in cancer cells present in blood serum. The sensors were nanowire-based field effect transistors (FETs) akin to those in computer chips. In FETs, a voltage applied to a tiny "gate" electrode controls the flow of charges between two other electrodes. Lieber and

CREDIT: G. ZHENG *ET AL.*, *NATURE BIOTECHNOLOGY* **23**, 10 (2005)

<<Sensitive. Nanowire devices can detect cancer proteins at femtomolar concentrations.

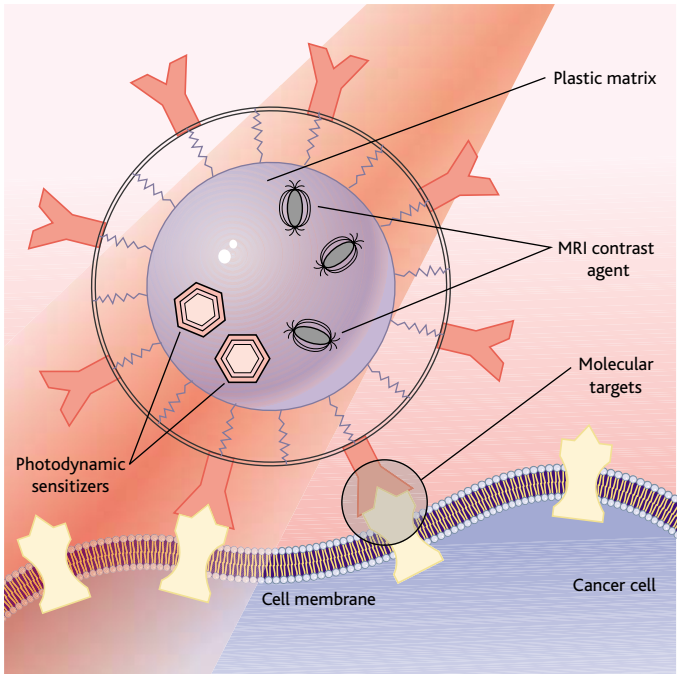
colleagues dotted charge-carrying silicon nanowires with monoclonal antibodies specific for the cancer proteins. When the proteins linked up with the antibodies, the electrical charges of the proteins changed the conductance of the silicon nanowires. This change signaled the presence and concentration of cancer markers. Lieber's team made devices that detected five cancer protein markers: prostate-specific antigen, PSA-alpha 1-antichymotrypsin, carcinoembryonic antigen, mucin 1, and telomerase.

The devices detected mere femtomolar concentrations of the target proteins without the fluorescent labels or complicated DNA-amplification procedures most often used to detect minute concentrations of biological compounds. What's more, the novel arrays contained 200 transistors that could be addressed individually, potentially opening the door to detecting scores of cancers by testing a single drop of blood.

Several other research teams have made similar progress in electrically detecting cancer-specific markers using other types of nanodevices. Last year, for example, researchers led by Hua Chen of NASA Ames Research Center in Mountain View, California, reported creating nanoelectrode arrays capable of electrically detecting single mutations in the *BRCA1* gene, which has been shown to predispose patients to several cancers including breast and ovarian cancers. First, the researchers took strands of DNA complementary to *BRCA1* DNA and bound them to electrodes made from carbon nanotubes. Then they poured a solution containing *BRCA1* over the arrays, which latched on to the target. By oxidizing nucleotides on the target, the researchers changed the conductivity of the nanotubes, which gave off a signal that could be picked up electronically.

Even further along are efforts to use tiny gold nanoparticles to help detect protein and DNA signatures for a number of diseases, including cancer. Two years ago, for example, Chad Mirkin and colleagues at Northwestern University in Evanston, Illinois, reported in *Science* a new protein-detecting technique up to a million times more sensitive than ELISA assays, the current standard (*Science*, 26 September 2003, p. 1884). The researchers start with gold nanoparticles, attach antibodies that specifically bind to proteins of interest, and tag the particles with readily identifiable DNA strands. If the target protein is in a test sample, the protein binds to the antibody on the nanoparticle. Next, the researchers add another target-seeking antibody tethered to a magnetic bead. They use a magnet to pull the beads—and everything bound to them—away from the rest of the sample, then identify their target protein by sequencing the DNA strands.

Mirkin says that clinical trials of the technique are planned for next year and that a biotech company that he co-founded called Nanosphere in Northbrook, Illinois, plans to commercialize diagnostics based on it within 2 years.



Triple threat. Multifunctional nanoparticles can combine tumor-seeking sensors, imaging agents, and toxins that kill cancer cells.

ILLUSTRATION: C. BICKEL/*SCIENCE*

In sight

Researchers are also making quick progress in using nanotechnology to spot cancer in its earliest stages inside the body. Last year, for example, researchers led by oncologist John Frangioni of Beth Israel Deaconess Medical Center in Boston reported that they had used semiconductor particles called quantum dots to image cancer cells in the sentinel lymph nodes of animals as large as pigs. The sentinel lymph nodes are typically the first to show signs of metastatic cancer cells shed by nearby organs. Oncologists check them for cancer through surgical biopsy—a tricky procedure, as the sentinel nodes are small and hard to locate.

Frangioni's group teamed up with quantum-dot experts led by Moungi Bawendi of the Massachusetts Institute of Technology in Cambridge. Bawendi's team synthesized nanosized onionlike structures composed of an inner cadmium-tellurium core surrounded by a cadmium-selenium layer and then capped with an organic compound to make the particles water-soluble. The particles are strong absorbers and emitters of infrared light. When the researchers injected animals with tiny amounts of the quantum dots, lymphatic cells quickly cleared the dots and routed them to the lymph nodes. As the researchers reported in the January 2004 issue of *Nature Biotechnology*, they could light up the lymph nodes even through centimeters of skin simply by shining near-infrared light from a halogen lamp. If the approach works in humans, it could guide surgeons to the lymph nodes of biopsy patients.

The notion of using cadmium-based quantum dots in humans has long come under fire, because the heavy metal is toxic. As an alternative, in the 3 August issue of the *Journal of the American Chemical Society*, the researchers reported creating indium-based semiconducting dots that also worked for mapping sentinel lymph nodes. These dots contained arsenic, another toxin, but the authors say the dose required to light up lymph nodes may be small enough to keep the toxicity low.

Infrared light–emitting nanoparticles are likely to prove most useful in spotting tumors near the skin surface. For tissues deep within the body, many groups are turning to magnetic nanoparticles that can be used as contrast agents for magnetic resonance imaging (MRI) machines. In May, for example, Carola Leuschner, a biochemist at the Pennington Biomedical Research Center in Baton Rouge, Louisiana, told attendees of the Nano Science and Technology Institute (NSTI) meeting in Anaheim, California, that her group has developed iron oxide nanoparticles capable of revealing the presence of breast cancer cells in mice. Leuschner's team targeted their iron oxide particles to tumor cells by covalently linking them to copies of a short peptide called LHRH, which seeks out and binds to receptors overexpressed on a wide variety of tumor cells. In mice inoculated with human breast cancer cells that caused them to develop tumors, the researchers imaged tumors just half a millimeter wide—far smaller than can be seen by conventional mammography and ultrasound techniques. "The potential for nanoparticles to improve tumor imaging is really very great," says Leuschner.

Search and destroy

Of course, finding cancer cells is only the first step. Nanotechnologists are developing a

number of particles designed to wipe out tumors as well. Many use targeting agents such as LHRH to direct toxic compounds to tumor cells. For example, Vladimir Torchilin and colleagues at Northeastern University in Boston are linking chemotherapeutic-containing nanoparticles to an antibody called 2C5, which homes in on the surface of human cancer cells. They have shown that the approach slows the growth of a variety of tumors, in part because the nanoparticles can ferry large amounts of the chemotherapeutic drugs to the tumor.

Moving to Market

Product	Type of nanomaterial	Indication	Phase	Company
VivaGel	Dendrimer	Topical microbicide for HIV	Phase I	StarPharma
MRX-952	Branching block copolymer self-assembled nanoparticulate formulation of irinotecan metabolite	Oncology	Preclinical	ImaRx Therapeutics
Abraxane	Nanoparticle albumin	Non–small cell lung cancer, breast cancer, others	NDA filed	American Pharmaceutical Partners
Cyclosert-camptothecin	Cyclodextrin nanoparticle	Metastatic solid tumors	IND filed	Insert Therapeutics
TNT AntiEpCAM	Polymer-coated iron oxide	Solid tumors	Preclinical	Triton BioSystems
Verigene platform	DNA-functionalized gold nanoparticles	Diagnostics	On market	Nanosphere
INGN-401	Liposome	Metastatic lung cancer	Phase I	Introgen
Combidex	Iron oxide nanoparticle	Tumor imaging	NDA filed	Advanced Magnetics

In the pipeline. Several nanotech cancer treatments are being tested or have been approved.

Other nanoparticle drugs take a less direct targeting approach. Because tumors grow so quickly, the blood vessels that form around them tend to be porous, leaking out small molecules around the tumor. Several groups hope the leakage will help them bombard tumors with tiny packages of toxins. Northeastern University pharmaceutical scientist Mansoor Amiji, for example, reported at the NSTI meeting that his team has loaded the anticancer compound paclitaxel (known more commonly by its trade name, Taxol) into tiny hollow polymer nanospheres, which release their cargo when exposed to the relatively low pH of tumor cells. Because the plastic spheres shield healthy cells from the drugs, the researchers can deliver higher concentrations of the drugs. In ongoing studies, Amiji reported, animals receiving the nanoparticle-based delivery systems have all survived longer than controls that received the drugs by themselves.

At the same meeting, Giulio Paciotti of CytImmune, a biotech company in Rockville, Maryland, reported a similarly effective strategy for delivering the highly toxic chemotherapeutic agent tumor necrosis factor to tumors by linking it to nanosized gold particles, which are good at escaping through leaky blood vessels. Because more of the drug accumulates in the target tissue, "it allows you to lower the dose and increase the safety," says Paciotti. "Nanoparticle drugs may block collateral damage so often due to chemotherapy," Downing says.

Jennifer West and Naomi Halas of Rice University in Houston, Texas, have pioneered another damage-control strategy in which they target tumors with gold-coated nanoparticles, which then become tiny heaters that cook tumor cells to death. To turn on the heat, the Rice researchers hit the nanoparticles inside tumors with infrared light. The light passes harmlessly through normal tissue, but the nanoparticles readily absorb it and warm up to more than 40°C. In the 11 November 2003 issue of the *Proceedings of the National Academy of Sciences*, the Rice researchers reported that their nanoscale heaters wiped out tumors in both cell culture and animal studies. Since then, other groups have reported similar success in heating tumors with carbon nanotubes and magnetic nanoparticles.

Such treatments, Ferrari notes, have a great potential to improve the safety of cancer treatment, because they kill cells only when they are activated by an external source.

Putting the pieces together

Nanotechnology's greatest advantage over conventional therapies may be the ability to combine more than one function. "Even though we think of nanoparticles as small, they are large compared to molecules. So you can decorate them with all kinds of bells and whistles to carry out multiple functions," Mirkin says. Chemist Raoul Kopelman of the University of Michigan, Ann Arbor, and colleagues, for example, have recently created three-component nanoparticles that target, image, and destroy tumors in the brains of rats. The particles consist of an iron oxide core that serves as an MRI contrast agent. Attached to them are copies of a cancer-targeting peptide called F3, as well as a light-absorbing compound called photofrin that kills cells when hit with red light. When Kopelman's team used their combination particles to treat rats previously injected with cancer cells inside their brains, animals receiving the combination nanoparticles survived more than twice as long as control animals receiving the nontargeted photofrin compound.

Another Michigan team, led by pathologist James Baker, achieved equally enticing results by targeting tumbleweedlike organic molecules called dendrimers designed to ferry large concentrations of traditional chemotherapeutic drugs and imaging agents inside cancer cells. Ferrari predicts that countless examples will come: "There are several thousand particle types and many vector types. What we are seeing is the tip of the iceberg."

Despite such progress, nanotechnology products face unique hurdles in making it to the clinic. Researchers must find ways to prevent immune cells from clearing nanoparticles before they reach their targets and must also overcome tumors' acquired ability to spit out cancer drugs that get inside cells. Even more challenging may be designing clinical trials for particles that perform more than one function. Trials for imaging agents are typically very different from those for drugs. "Do you have to design separate trials?" asks Downing. "We've been struggling with this."

A more general concern, Downing says, is the pharmaceutical industry's preference for blockbuster drugs with massive sales. "Cancer as a whole presents a challenge to the blockbuster drug model. It represents a fragmented market," Downing says. That prospect, he says, has slowed major pharmaceutical companies from jumping into nanotechnology research.

Finally, the toxicity of nanoparticles remains unclear. As a result, environmental health and safety agencies around the world continue to grapple with how best to regulate these novel materials (*Science*, 18 June 2004, p. 1732). "Those are very, very important concerns," Ferrari says. But over time, he says, patients will likely clamor for the novel therapies: "It is going to be very, very hard to come up with a nanoparticle drug that will be more toxic than the drugs out there today." If true, nano-based drugs will at least be less harmful than today's cancer fighters. But if they work as intended, they should also prove far more effective.

–ROBERT F. SERVICE

SOURCE: M. FERRARI AND G. DOWNING, *BIODRUGS* 19, 203 (2005)

Exploring and Engineering the Cell Surface Interface

Molly M. Stevens* and Julian H. George

Cells are inherently sensitive to local mesoscale, microscale, and nanoscale patterns of chemistry and topography. We review current approaches to control cell behavior through the nanoscale engineering of materials surfaces. Far-reaching implications are emerging for applications including medical implants, cell supports, and materials that can be used as instructive three-dimensional environments for tissue regeneration.

Deciding which protein to express, when to divide, when to specialize, and when to commit suicide are all ongoing processes that occur within cells. Genes must be activated in the correct sequence and synchrony in order to express the numerous proteins needed for proliferation and differentiation to hierarchical organization within organs. In addition to the intrinsic cell factors that regulate cell fate, extrinsic signals to the cell from the surrounding extracellular matrix (ECM) are essential in guiding it through distinct development paths.

Artificial biomaterial scaffolds designed to support cell and tissue growth have traditionally aimed on a macroscopic level to match the properties of the organs they are to replace without recreating the nanoscale detail observed in real organs (*1*). In the body, the nanoscale structure of the ECM provides a natural web of intricate nanofibers to support cells and present an instructive background to guide their behavior (*2*–*6*). Unwinding the fibers of the ECM reveals a level of detail unmatched outside the biological world. Each fiber hides clues that pave the way for cells to form tissues as complex as bone, liver, heart, and kidney. The ability to engineer materials to a similar level of complexity is becoming a reality (*1*, *7*–*12*). This review aims to highlight recent developments in biomaterials that have taken the lead from nanoscale engineering in nature to create more biomimetic cellular environments.

The Cell Extracellular Matrix

Cells are inherently sensitive to their surroundings. Typically between 10 and 100 μm in diameter, cells respond to environmental features at all length scales from the macro down to the molecular. The outer membrane of a typical cell is covered by specific carbohydrate structures and a forest of at least six different receptor systems that can be activated by interactions with adjacent cells, ligands in the surrounding ECM, and secreted signaling molecules. Hundreds of different proteins play a role in the composite stimulation of cell receptors, which in turn determine a plethora of responses, including cell migration in the early embryo, coordinated organogenesis, and wound repair throughout adult life (*2*–*4*, *6*). Collectively, these extrinsic factors make up a highly defined and specialized cell microenvironment, which is essential for correct tissue development and continued function.

The ECM takes a variety of forms in different tissues and at different stages of development in the same tissue (*3*, *13*). Diversity arises through combinations of specific molecular interactions between numerous isoforms, ratios, and geometrical arrangements of collagens, elastins, proteoglycans, and adhesion proteins such as fibronectins and laminins. This creates an environment that is replete with informational cues. In addition to this, a wealth of molecular mechanisms modulates the dissemination of this information. For example, the ECM plays a major role in regulating growth factor signaling, acting as a local reservoir for latent forms, and rapidly releasing and activating them on demand (*14*). Multiple motifs color each ECM protein. Encoded by specific amino acid sequences, these motifs target and bind specific cell surface receptors to trigger different intracellular signaling pathways. The transmembrane integrin receptors, with more than 20 members identified, are the most extensively characterized, and they recognize motifs such as Arg-Gly-Asp (RGD) within proteins of the ECM such as fibronectin and vitronectin (*15*). These receptors tether the cell cytoskeleton to the fibers of the ECM, forming local focal adhesions. When bound, integrins activate a cascade of intracellular signaling pathways, leading to changes in gene expression and affecting most aspects of cell behavior. They modify differentiation, proliferation, the further expression of ECM proteins, activation of growth factors, and the maintenance of survival signals to prevent apoptosis (programmed cell death) (*16*). Cell membrane receptors rarely act alone and frequently form part of multicomponent systems that allow for diverse signal integration, for example, between the growth factor and integrin signaling pathways (*17*, *18*).

Engineering ECM ligands, such as RGD, into artificial surfaces enhances functionality in terms of cell behavior. The cell's response is

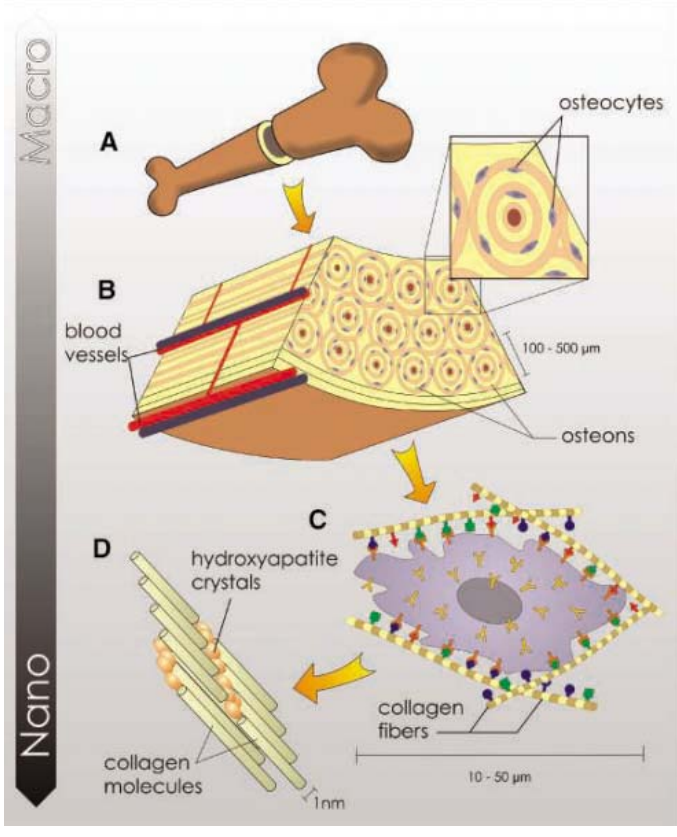


Fig. 1. Hierarchical organization of bone over different length scales. Bone has a strong calcified outer compact layer (**A**), which comprises many cylindrical Haversian systems, or osteons (**B**). The resident cells are coated in a forest of cell membrane receptors that respond to specific binding sites (**C**) and the well-defined nanoarchitecture of the surrounding extracellular matrix (**D**).

Department of Materials and Institute for Biomedical Engineering, Imperial College of Science, Technology, and Medicine, Prince Consort Road, London SW7 2BP, UK.

*To whom correspondence should be addressed. E-mail: m.stevens@imperial.ac.uk

often biphasic (e.g., migratory response) and always specific to particular ligand surface densities and binding affinities (*19*–*23*). This response can be modulated by close colocalization of synergistic ligands. For example, a spacing of 4 nm between RGD and the synergy site Pro-His-Ser-Arg-Asn (PHSRN) will lead to increases in indicators of osteoblast cell function, such as metabolic activity and alkaline phosphatase production, but also lead to decreases in ECM production (*20*). The importance of RGD ligand clustering as a prerequisite to certain intracellular signaling pathways has been demonstrated in vitro on two-dimensional (2D) surfaces at the nanometer scale (*15*, *22*). Control over ligand clustering and colocalization of multiple motifs (including growth factors) in 3D structures still poses challenges in materials design and synthesis. Nevertheless, advances in this area are likely to be highly relevant given that it is the precise spatial distribution of these motifs in tissues that determines many important aspects of cell behavior (*24*). Notably, the binding of integrins and the formation of focal adhesions, their structure, localization, and function in 3D tissues is substantially different from their binding and formation in 2D culture (*24*, *25*). The 3D ECM environment in vivo strongly influences changes in cell shape that affect the differentiation process (*13*, *26*). It is likely that cells require the full context of 3D nanofibrous matrix to maintain their phenotypic shape and establish natural behavior patterns.

Nanoscale Engineering at the Surface

Topographic reaction (i.e., reaction to the surface landscape) of cells to micrometer-range features such as grooves, ridges, and wells has been well established for decades (*27*). The fibers of the ECM and basement membrane (10 to 300 nm in diameter), their interconnecting nanopores, and hydroxyapatite crystals (4 nm) found in natural bone typically have nanoscaled dimensions (Fig. 1). Biomimetically driven studies are now exploring how the topography of a surface, if engineered with similar nanoscale structural features, can be used to control cell behavior.

Nanoscale alterations in topography elicit diverse cell behavior, ranging from changes in cell adhesion, cell orientation, cell motility, surface antigen display, cytoskeletal condensation, activation of tyrosine kinases, and modulation of intracellular signaling pathways that regulate transcriptional activity and gene expression (*27*). It is not only the scale of topography (5 nm to micrometer scale) that modulates cell behavior but also the type of ordered topography (e.g., ridges, steps, grooves, pillars, and pits) and even their symmetry (e.g., orthogonal or hexagonal packing of nanopits) (*28*–*35*). At present, there is great disparity between the experimental approaches taken by different groups, making it difficult to compare data on nominally similar systems.

A noticeable early response of the cell to nanotopography is to increase its complement of filopodia and microspikes (effectively the "sensing" organelles of the cell), which may heighten the cell's level of perception (*29*). In general, the presence of nanoscale features such as ridges, steps, and grooves increases cell attachment and proliferation, and ridges as thin as 70 nm guide cytoskeletal assembly (*28*, *31*). Certain cell phenotypes show greater sensitivity to particular nanoscale features than others. For example, osteoblasts have been found to adhere preferentially to carbon nanofiber compacts in competition with chondrocytes, fibroblasts, and smooth muscle cells (*30*). These cell-specific discrepancies serve to highlight one of the main challenges faced in understanding cell-material interactions: Conclusions drawn from one cell type cannot be readily applied to another.

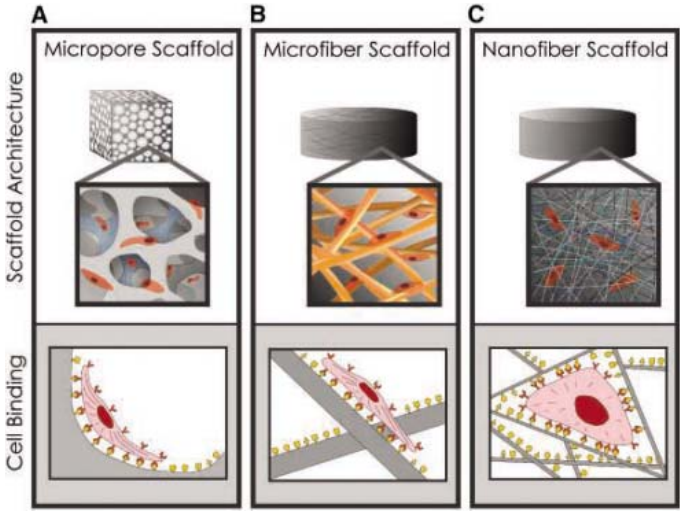


Fig. 2. Scaffold architecture affects cell binding and spreading. (**A** and **B**) Cells binding to scaffolds with microscale architectures flatten and spread as if cultured on flat surfaces. (**C**) Scaffolds with nanoscale architectures have larger surface areas to adsorb proteins, presenting many more binding sites to cell membrane receptors. The adsorbed proteins may also change conformation, exposing additional cryptic binding sites.

A powerful consequence of nanoscale engineering of the surface of materials is that topography alone can be used to elicit different responses from the same cell phenotype. For example, uroepithelial cells seeded on titanium surfaces engineered with well-defined nanometer topographies (hemispherical pillars or step edges) displayed modified cell morphologies and cytokine production to cells cultured on flat titanium controls with identical surface chemistry (*32*). The cells on the hemispherical pillars had a diminished release of the proinflammatory cytokine interleukin-6 and the chemokine interleukin-8, normally released in response to bacterial infection. In a separate study, the inclusion of nanoscale features in titanium through acid etching enhanced osteoblast differentiation and growth-factor production (*33*).

The question of how cells detect and respond to nanofeatures is as yet unresolved (*36*). Nanoscale topology likely modulates the interfacial forces that guide cytoskeletal formation and membrane receptor organization in the cell, which in turn can modify intracellular signaling (*29*). Nanoscale surface features may also affect the adsorption and conformation of integrin binding proteins, changing the availability of binding sites and modifying integrin signaling (*34*). Several studies have established that similar scale nanofeatures may elicit similar biological effects independent of the underlying material chemistry. For example, there is close agreement between smooth muscle cell behavior on both nanopatterned poly(methyl methacrylate) and poly(dimethylsiloxane), whereas the differing surface chemistry of the two polymers is unlikely to result in the same adsorption of proteins (*35*).

Nanoscale Scaffold Fabrication

The use of nanoscale material structuring to control cell behavior has important implications when designing new materials for tissue engineering. To regenerate tissue, engineered scaffolds play host to cells harvested from natural tissue. Conventionally, scaffolds have been designed macroscopically to have mechanical properties similar to natural tissue—hard scaffolds for bone (*10*) and elastic for bladder, veins, and arteries (*37*)—without the complexity and nanoscale detail observed in real organs at the level of the cell-matrix interaction. Approaches that progress toward the incorporation of this level of detail may provide real benefits (Fig. 2). Simply increasing the nanoscale roughness of the scaffold pore walls has already been found to increase cell attachment, proliferation, and expression of matrix components (*38*).

Combining nanostructured scaffolds and the incorporation of biological signals into the scaffold fabric is likely to prove most rewarding. This can be achieved by building scaffolds from naturally derived biopolymers such as elastin and collagen. These constructs have nanoscale structure and borrow their binding sites from the ECM biopolymers, providing innate informational guidance. However, maintaining the quality and activity of harvested biopolymers throughout their extraction, processing, and remodeling is challenging, and some voice concern over the direct use of animal-derived material in a medical setting.

Synthetic materials such as biodegradable polymers offer a versatile alternative to naturally

derived biopolymers. Their mechanical properties can be highly tailored and they are easy to synthesize and shape. Many synthetic polymers already have established medical histories and are routinely used in medical implants, substantially increasing their applicability for regulatory approval (*10*). Nanofibrous scaffolds have been successfully and relatively simply produced from many synthetic and natural polymers through the technique of electrospinning to produce fibers with diameters ranging from a few nanometers to micrometers (*39*, *40*). The nanofibers are continuous, potentially allowing for integrated manufacturing of 3D nanofiber matrices with high porosity, high spatial interconnectivity, and controlled alignment of fibers to direct cell orientation and migration (*41*, *42*). Given the diversity of tissue-specific orientation of fibrils (parallel and aligned in tendon, concentric weaves in bone, orthogonal lattices in cornea, and meshlike in skin), this latter feature is yet to be fully exploited. The "biological" fine-tuning of these scaffolds toward particular cell types is of growing interest. Once challenges in materials design and solvent compatibility have been overcome, bioactive composite and core-shell fibers may be engineered to deliver growth factors, peptides, enzymes, drugs, and even DNA (*42*–*44*).

Thermally induced phase separation is another approach based on the thermodynamic demixing of polymer solution into polymer-rich and -poor phases. It has been used to produce a wide range of spongelike scaffolds or, in some instances, polymer nanofibers that mimic the size and scale of natural collagen fibers (*45*, *46*). These new nanofibrous scaffolds promote cell attachment and proliferation and have been found to adsorb a wider spectrum and a greater quantity of integrin-binding proteins than do similar solid wall scaffolds (*45*).

Many natural biopolymers are assembled in multiple steps from the bottom up. The process of collagen self-assembly itself takes place over no fewer than nine separate steps (Fig. 3A). Synthetic nanoscale fabrication techniques cannot at present match nature's ingenious ability, but much can be learned from natural systems. A number of groups have developed rationally designed polypeptide systems that self-assemble into nanoscaled fibers in aqueous media (*47*), such as the self-assembly of amyloid-like fibers (*48*). A few modifications through rational de novo design can drive peptides with alternating hydrophobic and hydrophilic residues to associate into small, self-complementary β-sheet membranes. These further assemble into amyloid-like fibrils when suspended in an ionic solution of the correct pH (Fig. 3B) (*9*). Compared with electrospinning, the self-assembly approach can produce thinner fibrils, with diameters typically less that 10 nm, although these may cluster into thicker fiber bundles. When used in cell culture, the interwoven hydrogel structures physically surround cells in a manner similar to ECM and promote cell proliferation, active migration, and expression of ECM (*9*, *49*).

An illustration of an artificially designed, amphiphilic self-assembling scaffold uses macromolecules that have thin hydrophobic alkyl tails covalently bonded to thick hydrophilic peptide head-groups (*50*) (Fig. 3C). In aqueous solution, these assemble into long nanocylinders as the hydrophobic tails form a core to shield themselves from the aqueous solution. The resulting interwoven gel provides a previously unexplored approach to conductive bone regeneration. The peptide head groups include a phosphorylated serine, encouraging hydroxyapatite nucleation and an RGD motif to aid bone cell adhesion and survival within the resulting scaffolds. Included cysteine thiol residues covalently bond the head groups together. In effect, this system mimics the natural process by which collagen induces calcium, phosphate, and hydroxide to form hydroxyapatite crystallites within bone, which are necessary for bone's structural rigidity. Similar amphiphilic macromolecules that use the IKVAV domain are under investigation to create hydrogel scaffolds for neural cell differentiation (*51*).

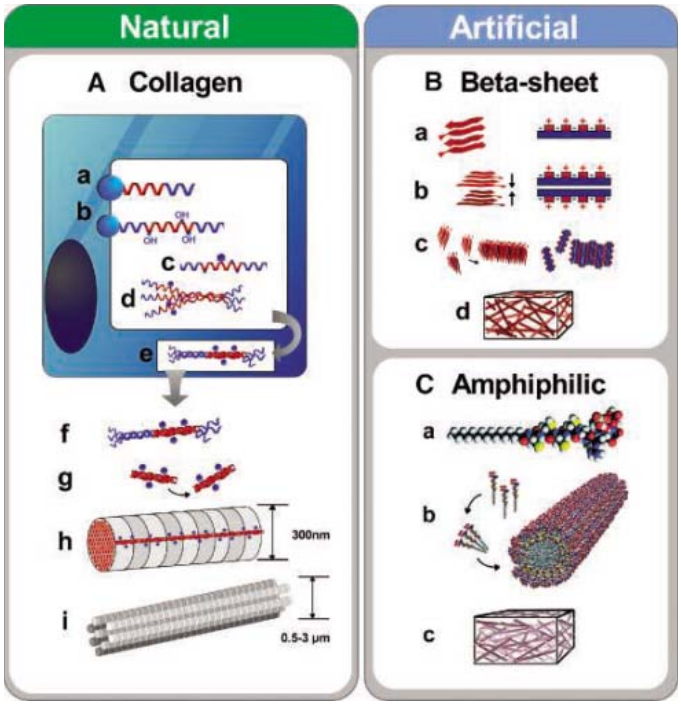


Fig. 3. Natural and artificial fiber self-assembly. (**A**) Natural assembly of collagen fibers: (a) Pro-alpha chains are synthesized in the lumen of the endoplasmic reticulum, where (b) hydroxylation of selected prolines and lysines occurs. (c) This is followed by glycosylation of selected hydroxylysines. (d) Three pro-alpha chains self-assemble and (e) form a procollagen triple helix. (f) These are secreted into the extracellular space, and (g) the protective propeptides are cleaved. (h) Finally, the procollagen self-assembles into fibrils, and (i) these aggregate into collagen fibers. (**B**) Amyloid-like assembly: (a) Beta-sheet membranes with hydrophilic and hydrophobic faces group by (b) burying their hydrophobic faces. In the correct ionic solution, (c) the hydrophilic faces attract and (d) the fibers self-assemble (*9*). (**C**) Amphiphilic peptide assembly: (a) Amphiphilic peptide design. (b) The peptides bury their hydrocarbon tails, (c) forming fibers in solution (*50*).

A Multidimensional Map

The many different types of information encoded into the extracellular environment combine to form a multidimensional map (Fig. 4). Cells use this map to guide their activities and maintain their differentiation within tissues. Changes in signaling gradient across the map provide directionally encoded signals. Cell receptors distributed across the cell membrane perceive these signal gradients and respond with directional behavior such as migration and the expression of matrix components (*52*, *53*). Different cells may even read the map in different ways, depending on their complement of receptors.

Each cell type fabricates its own network of precisely encoded ECM proteins to enable an exact tailoring of the structural properties and information content of each ECM environment throughout the body. As a consequence of the convoluted nature of protein folding, cryptic binding sites hidden within the structure of ECM molecules may be exposed through mechanisms that cleave or mechanically distort the proteins of the ECM (*54*, *55*) (Fig. 4). [For example, several ECM proteins such as laminin 5, collagen, and tenascin-C possess multiple repeats of weak affinity epidermal growth factor–like ligands that are revealed after ECM modification and can function as cellular promigratory tracks (*18*, *56*)]. As a result, information released dynamically by protein conformation change during tissue damage or deformation can direct cell behavior in response to these events. This unmasking of cryptic sites is a tightly controlled process signifying the importance of cryptic ECM functions. Through the dynamic response to external events and elaborate reciprocal flow of signaling between the ECM backdrop and the cells that express it, the behavior of cells is coordinated into complex functional tissues (*57*).

Repainting the Map

Engineering these dynamic ECM mechanisms into biomaterials offers further control over cell behavior. By tailoring proteolytic cleavage, cell

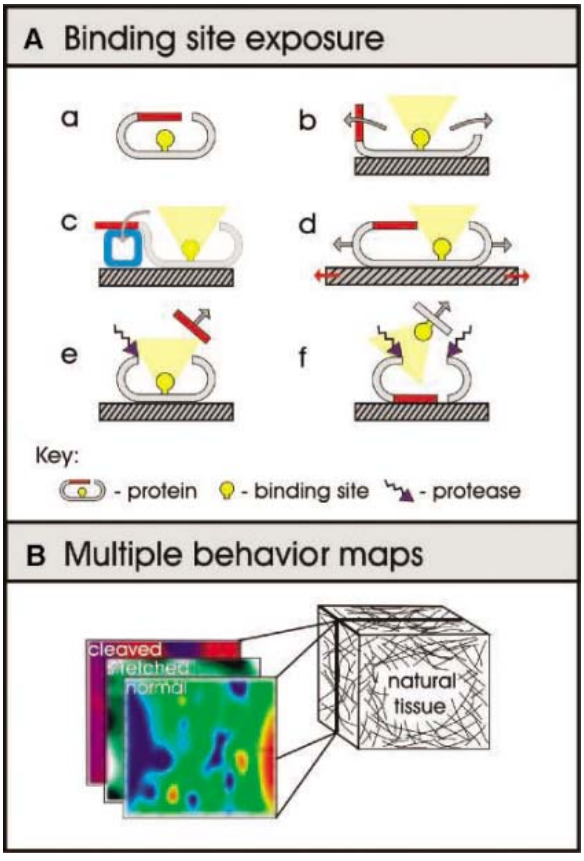


Fig. 4. Mechanisms of Cryptic Motif exposure. (**A**) (a) A representation of a protein structured with a hidden motif (yellow ball). Motifs can be exposed through conformation change due to (b) surface adsorption, (c) interaction with other proteins, and (d) mechanical distortion. (e) Proteolysis (purple arrow) can reveal hidden motifs and (f) may release them into the environment, enabling transport of signals away from the affected area. (**B**) A multidimensional tissue map. By providing different binding opportunities to cells in response to events that change protein conformation (such as stretching and cleaving), natural tissues effectively provide multiple behavioral maps, whose context is dependent on external events.

invasion can be encouraged and controlled. Designer oligopeptides that are recognized and cleaved by cell-secreted matrix metalloproteases have been successfully used to aid cell invasions into artificial poly(ethylene glycol)–based scaffolds (*58*) and to help induce in situ bone regeneration in combination with controlled release of recombinant human bone morphogenetic protein 2 (*59*). Elastin-like polymers, engineered with the use of recombinant DNA technologies, are also under investigation (*60*), with the aim of fabricating augmented fibrous scaffolds that promote cell invasion. As cells use proteolysis to migrate into these scaffolds, proteolytic fragments are released that present biologically active hexapeptide sequences to cell membrane receptors. Subsequent binding triggers signaling pathways that promote cell proliferation within the scaffold.

Future Directions

Nanostructuring of materials provides a powerful mechanism to encourage and direct cell behavior ranging from cell adhesion to gene expression. Early findings have been greeted with excitement from the orthopedic industries where the promotion of one cell type over another, such as osteoblasts (bone-forming cells) over osteoclasts (bone-resorbing cells), to stimulate bone growth will be important in reducing aseptic loosening and failure of implants (*10*). Other applications easily envisaged are for biotechnological culture supports, implanted biosensors (*61*), neural interfaces (*62*), and other structural medical implants engineered with nanoscale features to reduce the likelihood of fibrous encapsulation and allow stronger interfacing with the host tissue for a longer period of time.

A key challenge is to capture the degree of complexity that is needed to functionally replicate the ECM of natural tissue. To this end, nanostructured scaffolds enriched with specific proteins are likely to emerge as strong contenders for the biocomposites of choice for tissue regeneration. Nevertheless, we are still a long way from recreating the molecular architecture of the ECM and the dynamic mechanisms by which information is revealed in response to challenges within the local environment. Given the diversity of cryptic motifs discovered within the proteins of the ECM, facile structure-based elucidation of these mechanisms seems unlikely. Achieving effective temporal control over the signals that are presented to cells in 3D artificial matrices is still a key challenge in the optimization of outside-in signaling, as is the colocalization of cell signaling epitopes. Advances in the areas of fundamental matrix biology, nanofabrication, synthetic molecular self-assembly, recombinant DNA technologies (*63*), and printing technologies (*64*) will enable the generation of materials that can provide enhanced 3D tissue context maps of molecular and structural information.

References and Notes

1. B. D. Ratner, S. J. Bryant, *Annu. Rev. Biomed. Eng.* **6**, 41 (2004).
2. N. Zagris, *Micron* **32**, 427 (2001).
3. D. Gullberg, P. Ekblom, *Int. J. Dev. Biol.* **39**, 845 (1995).
4. M. Aumailley, B. Gayraud, *J. Mol. Med.* **76**, 253 (1998).
5. J. E. Scott, *J. Anat.* **187**, 259 (1995).
6. E. I. Wallner, Q. Yang, D. R. Peterson, J. Wada, Y. S. Kanwar, *Am. J. Physiol.* **275**, F467 (1998).
7. F. Rosso *et al.*, *J. Cell. Physiol.* **203**, 465 (2005).
8. H. Shin, S. Jo, A. G. Mikos, *Biomaterials* **24**, 4353 (2003).
9. S. Zhang, *Nat. Biotechnol.* **21**, 1171 (2003).
10. L. L. Hench, J. M. Polak, *Science* **295**, 1014 (2002).
11. M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **23**, 47 (2005).
12. D. G. Anderson, J. A. Burdick, R. Langer, *Science* **305**, 1923 (2004).
13. D. J. Behonick, Z. Werb, *Mech. Dev.* **120**, 1327 (2003).
14. J. Taipale, J. Keski-Oja, *FASEB J.* **11**, 51 (1997).
15. C. Bokel, N. H. Brown, *Dev. Cell* **3**, 311 (2002).
16. F. G. Giancotti, E. Ruoslahti, *Science* **285**, 1028 (1999).
17. G. E. Plopper, H. P. McNamee, L. E. Dike, K. Bojanowski, D. E. Ingber, *Mol. Biol. Cell* **6**, 1349 (1995).
18. K. T. Tran, L. Griffith, A. Wells, *Wound Repair Regen.* **12**, 262 (2004).
19. S. P. Palecek, J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, A. F. Horwitz, *Nature* **385**, 537 (1997).
20. D. S. Benoit, K. S. Anseth, *Biomaterials* **26**, 5209 (2005).
21. B. G. Keselowsky, D. M. Collard, A. J. Garcia, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5953 (2005).
22. G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells, L. G. Griffith, *J. Cell Sci.* **113**, 1677 (2000).
23. W. L. Murphy, K. O. Mercurius, S. Koide, M. Mrksich, *Langmuir* **20**, 1026 (2004).
24. E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, *Science* **294**, 1708 (2001).
25. B. M. Gumbiner, *Cell* **84**, 345 (1996).
26. D. E. Discher, P. Janmey, Y.-l. Wang, *Science* **310**, 1139 (2005).
27. A. Curtis, C. Wilkinson, *Biochem. Soc. Symp.* **65**, 15 (1999).
28. A. I. Teixeira, P. F. Nealey, C. J. Murphy, *J. Biomed. Mater. Res.* **71A**, 369 (2004).
29. A. S. Curtis *et al.*, *IEEE Trans. Nanobioscience* **3**, 61 (2004).
30. R. L. Price, K. Ellison, K. M. Haberstroh, T. J. Webster, *J. Biomed. Mater. Res. A* **70A**, 129 (2004).
31. R. L. Price, K. M. Haberstroh, T. J. Webster, *Med. Biol. Eng. Comput.* **41**, 372 (2003).
32. A.-S. Andersson *et al.*, *Biomaterials* **24**, 3427 (2003).
33. O. Zinger *et al.*, *Biomaterials* **26**, 1837 (2005).
34. T. J. Webster, L. S. Schadler, R. W. Siegel, R. Bizios, *Tissue Eng.* **7**, 291 (2001).
35. E. K. Yim *et al.*, *Biomaterials* **26**, 5405 (2005).
36. A. Curtis, *IEEE Trans. Nanobioscience* **3**, 293 (2004).
37. Y. Wang, G. A. Ameer, B. J. Sheppard, R. Langer, *Nat. Biotechnol.* **20**, 602 (2002).
38. M. A. Pattison, S. Wurster, T. J. Webster, K. M. Haberstroh, *Biomaterials* **26**, 2491 (2005).
39. J. A. Matthews, G. E. Wnek, D. G. Simpson, G. L. Bowlin, *Biomacromolecules* **3**, 232 (2002).
40. W. J. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan, F. K. Ko, *J. Biomed. Mater. Res.* **60**, 613 (2002).
41. Y. Dzenis, *Science* **304**, 1917 (2004).
42. Z. Ma, M. Kotaki, R. Inai, S. Ramakrishna, *Tissue Eng.* **11**, 101 (2005).
43. J. Zeng *et al.*, *Biomacromolecules* **6**, 1484 (2005).
44. Z. C. Sun, E. Zussman, A. L. Yarin, J. H. Wendorff, A. Greiner, *Adv. Mater.* **15**, 1929 (2003).
45. K. M. Woo, V. J. Chen, P. X. Ma, *J. Biomed. Mater. Res. A* **67**, 531 (2003).
46. F. Yang *et al.*, *Biomaterials* **25**, 1891 (2004).
47. M. G. Ryadnov, D. N. Woolfson, *Nat. Mater.* **2**, 329 (2003).
48. C. M. Dobson, *Nature* **426**, 884 (2003).
49. J. Kisiday *et al.*, *Proc. Natl. Acad. Sci. U.S.A* **99**, 9996 (2002).
50. J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **294**, 1684 (2001).
51. G. A. Silva *et al.*, *Science* **303**, 1352 (2004).
52. P. J. Van Haastert, P. N. Devreotes, *Nat. Rev. Mol. Cell Biol.* **5**, 626 (2004).
53. C. A. Parent, P. N. Devreotes, *Science* **284**, 765 (1999).
54. S. Schenk, V. Quaranta, *Trends Cell Biol.* **13**, 366 (2003).
55. F. X. Maquart, S. Pasco, L. Ramont, W. Hornebeck, J. C. Monboisse, *Crit. Rev. Oncol. Hematol.* **49**, 199 (2004).
56. C. S. Swindle *et al.*, *J. Cell Biol.* **154**, 459 (2001).
57. C. D. Roskelley, A. Srebrow, M. J. Bissell, *Curr. Opin. Cell Biol.* **7**, 736 (1995).
58. M. P. Lutolf *et al.*, *Proc. Natl. Acad. Sci. U.S.A* **100**, 5413 (2003).
59. M. P. Lutolf *et al.*, *Nat. Biotechnol.* **21**, 513 (2003).
60. A. Girotti *et al.*, *J. Mater. Sci. Mater. Med.* **15**, 479 (2004).
61. J. T. Santini, M. J. Cima, R. Langer, *Nature* **397**, 335 (1999).
62. A. Curtis, *Nature* **416**, 274 (2002).
63. J. C. van Hest, D. A. Tirrell, *Chem. Commun. (Camb.)* 1897 (2001).
64. V. Mironov, T. Boland, T. Trusk, G. Forgacs, R. R. Markwald, *Trends Biotechnol.* **21**, 157 (2003).
65. We thank R. Langer and L. Hench for constructive comments on the manuscript.

10.1126/science.1106587

Tissue Cells Feel and Respond to the Stiffness of Their Substrate

Dennis E. Discher,[1]* Paul Janmey,[1] Yu-li Wang[2]

Normal tissue cells are generally not viable when suspended in a fluid and are therefore said to be anchorage dependent. Such cells must adhere to a solid, but a solid can be as rigid as glass or softer than a baby's skin. The behavior of some cells on soft materials is characteristic of important phenotypes; for example, cell growth on soft agar gels is used to identify cancer cells. However, an understanding of how tissue cells—including fibroblasts, myocytes, neurons, and other cell types—sense matrix stiffness is just emerging with quantitative studies of cells adhering to gels (or to other cells) with which elasticity can be tuned to approximate that of tissues. Key roles in molecular pathways are played by adhesion complexes and the actin-myosin cytoskeleton, whose contractile forces are transmitted through transcellular structures. The feedback of local matrix stiffness on cell state likely has important implications for development, differentiation, disease, and regeneration.

Anchorage dependence refers to a cell's need for adhesion to a solid. Most tissue cells are simply not viable upon dissociation and suspension in a fluid, even if soluble proteins are added to engage cell adhesion molecules [e.g., integrin-binding RGD peptide (*1*, *2*)]. Fluids are clearly distinct from solids in that fluids will flow when stressed, whereas solids have the ability to resist sustained pushing and pulling. In most soft tissues—skin, muscle, brain, etc.—adherent cells plus extracellular matrix contribute together to establish a relatively elastic microenvironment. At the macro scale, elasticity is evident in a solid tissue's ability to recover its shape within seconds after mild poking and pinching, or even after sustained compression, such as sitting.

At the cellular scale, normal tissue cells probe elasticity as they anchor and pull on their surroundings. Such processes are dependent in part on myosin-based contractility and transcellular adhesions—centered on integrins, cadherins, and perhaps other adhesion molecules—to transmit forces to substrates. A normal tissue cell not only applies forces but also, as reviewed here, responds through cytoskeleton organization (and other cellular processes) to the resistance that the cell senses, regardless of whether the resistance derives from normal tissue matrix, synthetic substrate, or even an adjacent cell. Furthermore, physical properties of tissues can change in disease [as imaged now by magnetic resonance imaging (MRI) or ultrasound elastography (*3*–*5*)], and cellular responsiveness to matrix solidity can likewise change, as illustrated by the growth of cancer cells on soft agar [e.g., (*6*)].

Contractile forces in cells are generated by cross-bridging interactions of actin and myosin filaments. For adherent cells, some of these forces are transmitted to the substrate (referred to as traction forces) and cause wrinkles or strains when the substrate consists of a thin film or a soft gel (*7*–*12*) (Fig. 1A). The cell, in turn, is shown to respond to the resistance of the substrate, by adjusting its adhesions, cytoskeleton, and overall state. Although considerable attention has been directed at the responsiveness of individual cells to external forces (outside→in) that range from fluid flow to direct stretching and local twisting (*13*), we are now beginning to understand that cellular responses to cell-exerted forces involve a feedback loop of inside→outside→in that couples to the elasticity of the extracellular microenvironment. An analogy to muscle building is perhaps useful: A bicep is not built by passive flexing; the muscle must do active work against a load. Moreover, a load of 1 kg clearly feels different from a load of 2 kg. Similar sensitivity, growth, and remodeling principles seem to apply to most anchored cells.

On ligand-coated gels of varied stiffness, epithelial cells and fibroblasts (*14*) were the first cells reported to detect and respond dis-

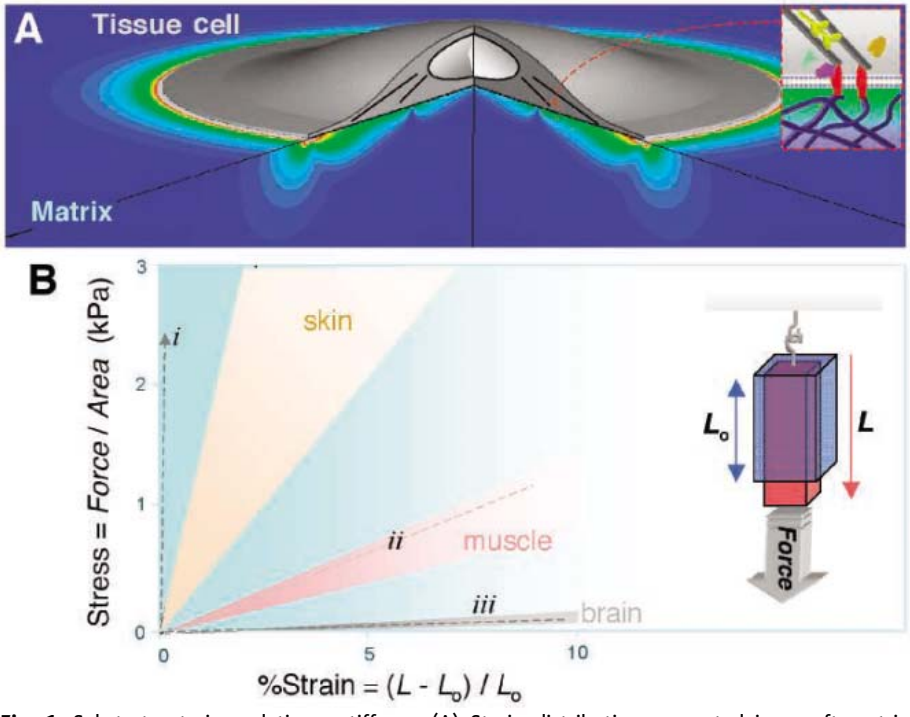


Fig. 1. Substrate strain and tissue stiffness. (**A**) Strain distribution computed in a soft matrix beneath a cell. The circular cell has a uniform and sustained contractile prestress from the edge to near the nucleus (*81*). (**B**) Stress versus strain illustrated for several soft tissues extended by a force (per cross-sectional area). The range of slopes for these soft tissues subjected to a small strain gives the range of Young's elastic modulus, *E*, for each tissue (*24*, *28*, *30*). Measurements are typically made on time scales of seconds to minutes and are in SI units of Pascal (Pa). The dashed lines (---) are those for (i) PLA, a common tissue-engineering polymer (*89*); (ii) artery-derived acellularized matrix (*90*); and (iii) matrigel (*42*).

[1]School of Engineering and Applied Science and Cell and Molecular Biology Graduate Group, University of Pennsylvania, Philadelphia, PA 19104–6315, USA. [2]Departments of Physiology and Cell Biology, University of Massachusetts, Worcester, MA 01655, USA.

*To whom correspondence should be addressed. E-mail: discher@seas.upenn.edu

tinctly to soft versus stiff substrates. Although molecular pathways are still only partially known, muscle cells, neurons, and many other tissue cells have since been shown to sense substrate stiffness (*15*–*17*). Unlike cells on soft gels or in tissues, cells cultured on tissue-culture plastic or glass coverslips are attached (often via adsorbed matrix protein) to essentially rigid materials. The question therefore arises: Do cells perceive and respond to the rigidity of these conventional materials in ways that contrast with their behavior in much more compliant tissues, gels, or sublayers of cells? The increasingly clear, affirmative answer to this question appears important in its impact not just on standard cell culture but also, perhaps, in understanding disease processes, morphogenesis, and tissue-repair strategies.

Soft Tissue Benchmarks

Cells adhere to solid substrates that range in stiffness from soft to rigid and that also vary in topography and thickness (e.g., basement membrane). Regardless of geometry, the intrinsic resistance of a solid to a stress is measured by the solid's elastic modulus E, which is most simply obtained by applying a force—such as hanging a weight—to a section of tissue or other material and then measuring the relative change in length or strain (Fig. 1B, inset). Another common method to obtain E involves controlled poking by macro- and micro-indenters, including atomic force microscopes (AFMs) (*18*, *19*). Many tissues and biomaterials exhibit a relatively linear stress versus strain relation up to small strains of about 10 to 20%. The slope E of stress versus strain is relatively constant at the small strains exerted by cells (*20*), although stiffening (increased E) at higher strains is the norm (*21*, *22*). Nonetheless, microscopic views of both natural and synthetic matrices [e.g., collagen fibrils and polymer-based mimetics (*23*)] suggest that there are many subtleties to tissue mechanics, particularly concerning the length and time scales of greatest relevance to cell sensing. Sample preparation or state is another obvious issue; for example, elastic moduli of whole brain in macroscopic measurements can vary by a factor of 2 or more, depending on specifics of preparation, tissue perfusion, etc. (*24*). In addition, with cells as well as tissues, many probing methods involve high-frequency stressing (*25*), whereas relevant time scales for cell-exerted strains seem likely to range from seconds to hours, motivating long time studies of cell rheology [recent cell mechanics references (*26*, *27*)]. Regardless, comparisons of three diverse tissues that contain a number of different cell types show that brain tissue is softer than muscle (*28*, *29*), and muscle is softer than skin (*30*) (Fig. 1B). Although mapping soft tissue micro-elasticities at a resolution typical in histology seems important, the implication here is that there are distinct elastic microenvironments for epithelial cells and fibroblasts in skin, for myotubes in fiber bundles, and for neurons in brain.

Correlations have long been made between increased cell adhesion and increased cell contractility [e.g., (*31*)], but it now seems clear that tactile sensing of substrate stiffness feeds back on adhesion and cytoskeleton, as well as on net contractile forces, for many cell types. Seminal studies on epithelial cells and fibroblasts exploited inert polyacrylamide gels with a thin coating of covalently attached collagen (*14*). This adhesive ligand allows the cells to attach and—by controlling the extent of polymer cross-linking in the gels—E can be adjusted over several orders of magnitude, from extremely soft to stiff. Images of adhesion proteins such as vinculin are revealing (Fig. 2, top): Soft, lightly cross-linked gels ($E \sim 1$ kPa) show diffuse and dynamic adhesion complexes. In contrast, stiff, highly cross-linked gels ($E \sim 30$ to 100 kPa) show cells with stable focal adhesions, typical of those seen in cells attached to glass. Similarly, rigidification of cell-derived three-dimensional (3D) matrices alters 3D-matrix adhesions, because the adhesions are replaced by large, nonfibrillar focal adhesions similar to those found on fixed 2D substrates of fibronectin (*32*). Consistent with a role for signaling in stiffness sensing, tyrosine phosphorylation on multiple proteins (including paxillin) appears broadly enhanced in cells on stiffer gel substrates (*14*), whereas pharmacologically induced, nonspecific hyperphosphorylation drives focal adhesion formation on soft materials. Inhibition of actomyosin contractions, in contrast, largely eliminates prominent focal adhesions, whereas stimulation of contractility drives integrin aggregation into adhesions (*33*). Additionally, although microtubules have been proposed to act as "struts" in cells and thus limit wrinkling of thin films by cells (*34*), quantification of their contributions to cells on gels shows that they provide only a minor fraction of the resistance (14%) to contractile tensions; most of a cell's tension is thus resisted by matrix (*35*).

Traction stresses (τ, force per area) exerted by fibroblasts on gels were the first to be mapped by embedding fluorescent microbeads near the gel surface and then imaging bead displacements before and after cell detachment (*10*, *20*). Although larger tractions are exerted on stiffer gels, typical tractions of $\langle\tau\rangle \sim 1$ kPa exceed by orders of magnitude the viscous fluid drag on any cell crawling in culture. In addition, mean cell tractions equate to mean gel strains that differ very little ($\varepsilon_{out} = \langle\tau/E\rangle \cong$ 3 to 4%) between gels that differ by twofold in E. This suggests that ε_{out} is sensed by cells as a tactile set-point, perhaps analogous to other physiological set-points such as extracellular ion concentrations or optimal growth factor concentrations. Furthermore, if matrix strain is approximately constant, then cells on soft gels need be less contractile than on stiff gels, and if they are less contractile, then

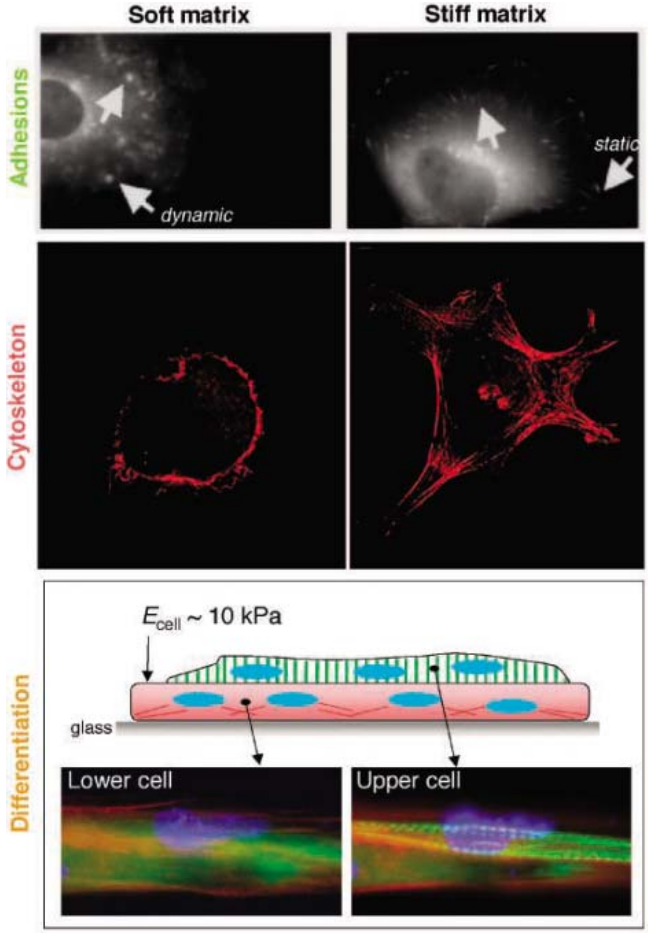


Fig. 2. Substrate stiffness influences adhesion structures and dynamics (*14*), cytoskeleton assembly and cell spreading (*17*, *42*), and differentiation processes such as striation of myotubes (*28*). (Top) The arrows point to dynamic adhesions on soft gels and static, focal adhesions on stiff gels. [Adapted from (*14*)] (Middle) The actin cytoskeleton. (Bottom) A cell-on-cell layering in which the lower layer is attached first to glass so that the upper layer, which fuses from myoblasts that are added later, perceives a soft, cellular substrate.

their adhesions need not be as strong. This is consistent with a reduced adhesion strength as measured by reduced forces to peel cells from soft gels versus glass (*28*). This is also consistent with more dynamic adhesions on soft substrates (Fig. 2, top). Fluorescence imaging also shows increasingly organized F-actin and stress fibers on increasingly stiff substrates in fibroblasts (Fig. 2, middle). Neurons, in contrast, appear to apply very little stress to their substrate, because they can only deform very soft gels (*36*). Neurons also branch more on softer substrates (*37*), perhaps because the cytoskeleton is more pliable, if less structured.

Differentiation and a Cell-on-Cell Hypothesis

Cytoskeletal organization in muscle cells also depends on substrate stiffness and reveals an optimal substrate stiffness for striation of actomyosin (*28*, *38*)—the contractile element of the myotube. On very soft gels that are micropatterned with collagen strips so as to generate well-separated myotubes, actomyosin appears diffuse after weeks in culture. On very stiff gels, as well as on glass micropatterns, stress fibers and strong focal adhesions predominate, suggesting a state of isometric contraction. Notably, however, on gels with an elasticity that approximates that of relaxed muscle bundles ($E \sim 10$ kPa), a large fraction of myotubes in culture exhibit definitive actomyosin striations. Actomyosin striation is even more prominent when cells are cultured on top of a first layer of muscle cells (Fig. 2). The lower myotubes attach strongly to glass and form abundant stress fibers, whereas the upper myotubes differentiate to the more physiological, striated state. Although cell-cell contact may provide additional signals, the elasticity E of the myotubes, as measured by atomic force microscopy, is in the same range as that of gels optimal for differentiation and—importantly—in the same range as that of normal muscle tissue.

Cell-cell contact appears to induce similar cell-on-gel effects for systems other than muscle. Astrocytes growing on glass, for example, appear to provide a soft cell "stroma" adequate for neuronal branching that is similar to gels having brainlike E (*39*). Cell-cell contact may have a similar effect when cells are grown at a high density. When endothelial cells are confluent, the cells have indistinguishable morphologies on soft versus stiff substrates (*40*), whereas cells attached only to an underlying stiff surface differ in their spreading and cytoskeletal organization (Fig. 2). Related results are also emerging with epithelial cells and fibroblasts, as well as cardiomyocytes that show a tendency to aggregate and form cell-cell contacts in preference to contact with soft gels (*41*). Such studies may set the stage for a better understanding of mechanosensitivity in cell-cell interactions during embryogenic and tissue regeneration processes.

Materials ranging from fibrin gels and microfabricated pillars to layer-by-layer polymer assemblies (*41*–*45*) all suggest a similar trend of more organized cytoskeleton and larger, more stable adhesions with increasing E as outlined here, despite likely differences in adhesive ligand density and long-time elasticity. However, the responses appear to be specific to anchorage-dependent and/or relatively contractile cells. Highly motile amoeboid cells such as human neutrophils are perfectly viable in blood (a fluid) and do not appear to be sensitive to substrate stiffness; neutrophils spread on soft gels just as much as they do on stiff gels and glass, whether activated or not (*46*–*48*). Although additional study is needed and could prove ligand dependent, the initial contrast with cells derived from solid tissue highlights the compelling need for insights into molecular pathways of stiffness sensing in relation to anchorage dependence and contractility. Variation with cell type implies an active, regulated response, rather than a universal need of cells to exert traction forces on a stiff matrix. Differences no doubt depend in part on expression and engagement of adhesion molecules. Integrins reportedly undergo adhesion-modulating conformational changes in response to force (*49*), and they also appear to be down-regulated on soft gels [e.g., α_5-integrin (*40*)]. However, overexpression of α_5-integrin does not override the limited spreading of cells on soft gels, whereas overexpression of actin drives cytoskeletal assembly and strongly promotes spreading (*17*).

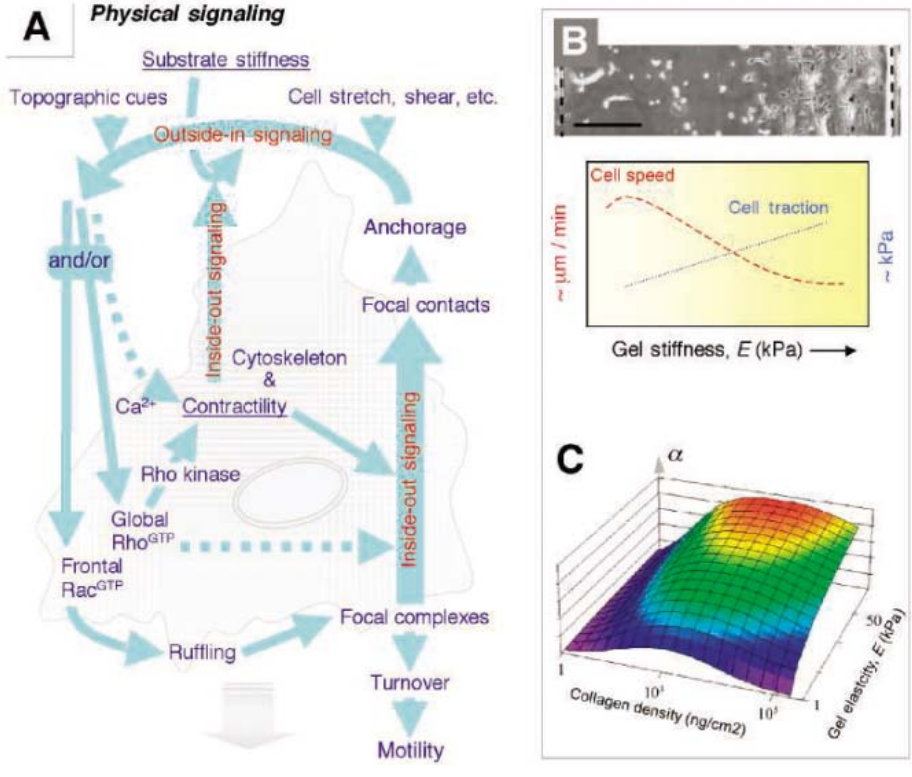


Fig. 3. Substrate stiffness influences contractility, motility, and spreading. (**A**) Interplay of physical and biochemical signals in the feedback of matrix stiffness on contractility and cell signaling as extended from (*91*). (**B**) Cells exert less tension on softer, collagen-coated gels but crawl faster (*20*), causing an accumulation of cells toward the stiff end of a soft-to-stiff gradient gel (*54*). Curves are schematic. [Image adapted from (*54*)] (**C**) Spread area, α, of smooth muscle cell versus ligand density and matrix stiffness, based on measurements fitted by a thermodynamic model (*17*). Similar nonlinear responses are also seen for adhesions, cytoskeleton organization, tractions exerted on the substrate, and other cellular processes.

Nonlinear Response to Compliance Signals and Molecular Effectors

Myosin inhibitors—including a potent nonmuscle myosin II inhibitor, blebbistatin (*50*)—have provided key evidence for the critical role of contractility in substrate sensing (*14*, *38*). Important roles are also reported for integrating activator proteins of the Ras superfamily, especially Rho subfamily members that are broadly known to regulate the cytoskeleton, cell growth, and transcription. In cells such as fibroblasts, it is well established that Rho-stimulated contractility drives stress fiber and focal adhesion formation and that up-regulation of α smooth muscle actin correlates with contractility on rigid substrates (*33*, *51*). Rac1 is another Rho family protein that when activated in macrophages, promotes engulfment of antibody-bearing soft beads, which otherwise are not engulfed (*48*). RhoA,

in contrast, has no observable effect in these measurements. Current views of signaling pathways, especially various physical signals (Fig. 3A), clearly implicate Rac in cell motility (versus contractility)—indeed, myosin inhibition activates Rac (*52*). The involvement of contractile-effector proteins in sensing implies that cell crawling is also likely to be sensitive to substrate stiffness (Fig. 3B), as demonstrated in studies of the "cell on gel" effect with epithelial cells (*14*), fibroblasts (*20*), and smooth muscle cells (*53*, *54*). With the latter cell type, crawling speed appears maximal at an intermediate stiffness. The result is reminiscent of a bell-shaped curve of crawling speed versus the concentration of adhesive ligand (*55*), which has been mathematically modeled as a shift in the balance between ligand-mediated traction and ligand-mediated anchorage (*56*). Additionally, smooth muscle cells on gels are slowed by inhibition of Rho kinase, suggesting that RhoA activity contributes to the tensions needed to detach any established adhesions at the rear of a motile cell (a process not needed in engulfment) (*57*). The dependence of cell crawling speed and direction on substrate stiffness, particularly gradients in stiffness, is now referred to as "durotaxis" (*20*).

Molecular mechanisms involved in cellular responses to matrix stiffness are still open to investigation, but it seems important to consider close relationships (or not) between "inside→outside→in" pathways and "outside→in" pathways (Fig. 3A). Adhesions on stiff materials are multifaceted mechanosensors [for a review, see, e.g., (*5*)], and, on the one hand, contractility does appear to regulate the formation and dynamics of adhesion structures (*14*). Indeed, myosin II has a well-established role on rigid substrates in adhesion and cytoskeletal organization (*33*), as well as spreading (*58*) and cell tension (*13*). On the other hand, applying external forces to cells (outside→in) leads to growth of focal adhesions on rigid materials, with or without myosin contractile forces (*59*). Nonetheless, inside→outside activity can trigger outside→in pathways such as the opening of stress-activated channels (*60*), with induction of calcium transients and activation of calmodulin and myosin II.

Additional work from the outside→in perspective has shown that stretching well-spread cells leads to deactivation of Rac (for <30 min) without affecting Rho activity (*52*). Stretching can also create new cytoskeletal binding sites for activator and adapter proteins (*61*) and thus alter the balance between protrusion and contractility. The mechanism may involve conformational changes to uncover scaffold binding sites or other activities; for example, one key focal adhesion protein, talin, must unfold for vinculin binding (*62*–*64*), and although the unfolding forces are not yet clear, similar helical bundle cytoskeletal proteins unfold at forces that just a few myosin molecules can generate. On the other hand, fluid shearing of endothelial cells activates Rho and also increases cell traction forces (*65*), but how such stimulation—transient or sustained—depends on myosin activity and compares with substrate-mediated pulling forces or substrate compliance effects remains unknown.

The effects and effectors of contractility can be transient as well as nonlinear, but are nonetheless essential to clarify. The temporary deactivation of Rac with stretch may have to be integrated over time to understand its place in signaling (*66*), and although myosin II activity is crucial for stiffness sensing, on rigid substrates it only delays the earliest phase of cell spreading (by ~2 hours), apparently through stiffening of the cell cortex (*67*). Overstimulation of myosin, like overstimulation of most motors, is also likely to slow and eventually stall cell migration. The effect may be related to the formation of less dynamic myosin assemblies on progressively stiffer substrates, fostering larger, more stable focal adhesions. Reconstitution experiments with mixtures of actin, myosin, adenosine 5′-triphosphate (ATP), and cross-linkers might lend important insight into motor-driven self-assembly processes.

Varied responses to mechanical signals at the cellular and molecular scales are increasingly in need of multivariate analyses. More data are needed to define coupled responses to substrate stiffness, contractile state, ligand density, and activator levels, as well as effects such as growth factor stimulation. A number of studies have already revealed nonlinear response maps, as illustrated by the spread area of cells on gels (Fig. 3C). Modeling efforts to date have been thermodynamic (*17*, *68*), kinetic (*56*), and—for cell-cell interactions—purely mechanical (*69*), but all generally yield nontrivial responses, saturable or even bell-shaped in E and other inputs. A major challenge in all such modeling is to clarify the principal enigma: how contractile traction forces exerted by a cell tend to increase with stiffness of the cell's substrate.

Do Cells Feel Their Way in Organogenesis?

Cell type–dependent increases in contractility with increasing substrate stiffness may offer partial answers to some long-standing questions of cell-cell organization. Random mixtures of two cell types often lead to shell-core cell aggregates (Fig. 4), as first observed when heart cells segregated into the interior of a mass of retinal cells after 1 day in culture (*70*). Individual cell clusters form by "pulling" away from each other (*71*). Such observations are now being used to manipulate aggregate morphologies through printing of cell masses into gels as toroids and other shapes (*72*). Such phenomena have been explained by a "differential adhesion hypothesis" in which different cell types bear different numbers and types of adhesion proteins (e.g., cadherins), giving rise to an effective surface tension, γ, at interfaces with cell layers (*73*). Although possible contributions of cytoskeleton and cell tension have not yet been reported, studies of zebrafish embryos (*74*) have shown that (i) disruption of actin filaments dissociates cells entirely, even though cadherins remain at the cell surface; and (ii) the effect is potentiated by at least one drug that inhibits actomyosin contractility.

Quantitative estimates of γ for the spherical aggregates of cadherin-expressing cells (*73*) exceed the rate-dependent cohesive strength of lipid bilayers [as low as 2 to 3 mN/m (*75*)] and suggest adhesion energies per cadherin that are orders of magnitude larger than would be expected of individual cadherin bonds. Such large γ values could be due to the cytoskeleton or even contractility (because $\gamma/\langle\tau\rangle \cong 1$ to 10 μm is a stress fiber length scale), especially because there is growing evidence of common RhoGTPase-cytoskeleton signaling among integrin- and cadherin-mediated adhesion (*76*–*79*). A major role for contractility in cell sorting was speculated long ago (*80*), but results reviewed here make it clear that contractile state can be strongly influenced by

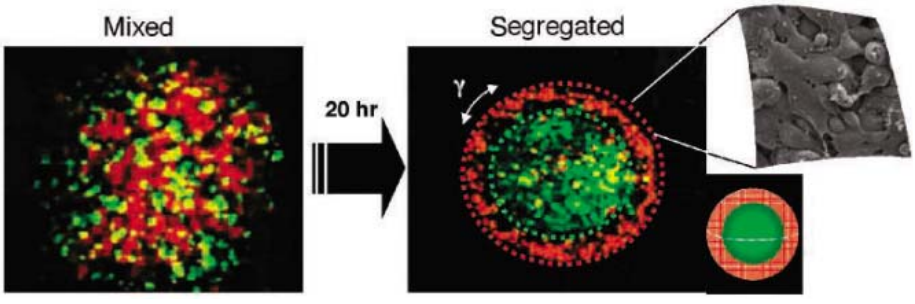


Fig. 4. Sorting of two cell types into a 3D shell-core aggregate (~300 μm in diameter) in which low expressers of N-cadherin (labeled in red) surround high expressers of N-cadherin (labeled in green) (*73*). Scanning electron micrograph of a typical spheroid's surface shows well-spread cells. [Adapted from (*73*) with permission from Elsevier. Image courtesy of G. Forgacs, University of Missouri]

the stiffness of the anchoring substrate. Heart cells pulling on equally stiff heart cells can generate a positive and steady feedback on their cytoskeleton that may not occur when these cells pull on other tissue cell types. Cell aggregation of less differentiated cells such as some stem cells that assemble into "embryoid bodies" has yet to be studied with myosin inhibitors or related methods, but the principles may extend to stem cell differentiation, particularly because at least some stem cells express nonmuscle myosin II at levels similar to those of myoblasts (*81*).

Added Facets and Prospects

Mechanobiology is a broad field. Emphasized here is the recent recognition that most tissue cells not only adhere to but also pull on their microenvironment and thereby respond to its stiffness in ways that relate to tissue elasticity. Many emerging topics are not dealt with adequately in this brief review of substrate stiffness effects. These include in vitro models of fibrotic stiffening and related disease processes (*82*, *83*); perturbed secretion and uptake (*84*, *85*); 2D versus 3D responses (*32*, *86*); deformations of fibronectin and other matrix molecules (*87*); structure formation such as capillary development (*15*, *88*); deeper aspects of cell differentiation such as with stem cells (*81*); the relative sensitivity and contractility of some cells relative to others; and broader effects of matrix elasticity, as well as fluidity (i.e., matrix rheology), on cells in tissue development, remodeling, and regeneration. For the cell biologist, this review may suggest the need for a better understanding of mechanochemical pathways and the benefit of more biologically relevant elastic substrates than rigid coverslips and polystyrene for in vitro studies. For the applied biologist or bioengineer, modified strategies for tissue repair and cell scaffolding may emerge, such as the development of fibrous scaffolds for cell seeding (*23*), where careful attention can be given to fiber flexibility. All of these topics seem likely to add to our rapidly growing recognition that tissue cells feel and respond to the mechanics of their substrate in many contexts.

References and Notes

1. E. Ruoslahti, M. D. Pierschbacher, *Science* **238**, 491 (1987).
2. H. L. Hadden, C. A. Henke, *Am. J. Respir. Crit. Care Med.* **162**, 1553 (2000).
3. For example, classic palpation self-exam reveals that tumor stiffening can be quantitated by both MRI (*4*) and ultrasound elastography (*5*).
4. A. L. McKnight *et al.*, *AJR Am. J. Roentgenol.* **178**, 1411 (2002).
5. J. Bercoff *et al.*, *Ultrasound Med. Biol.* **29**, 1387 (2003).
6. D. R. Welch *et al.*, *Cancer Res.* **60**, 1552 (2000).
7. A. K. Harris, P. Wild, D. Stopak, *Science* **208**, 177 (1980).
8. Since (*7*), methods for measuring traction force have become more quantitative by embedding beads in either films (*9*) or gels (*10*) or by using deformable patterns (*11*) or posts (*12*).
9. T. Oliver, M. Dembo, K. Jacobson, *J. Cell Biol.* **145**, 589 (1999).
10. W. A. Marganski, M. Dembo, Y. Wang, *Methods Enzymol.* **361**, 197 (2003).
11. N. Q. Balaban *et al.*, *Nat. Cell Biol.* **3**, 466 (2001).
12. J. L. Tan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1484 (2003).
13. F. J. Alenghat, D. E. Ingber, *Sci. STKE* **119**, pe6 (2002).
14. (Fibroblasts and epithelial cells) R. J. Pelham, Y. Wang. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13661 (1997) with Erratum **95**, 12070a (1998).
15. (Endothelial cells) C. F. Deroanne, C. M. Lapiere, B. V. Nusgens., *Cardiovasc. Res.* **49**, 647 (2001).
16. (Transformed cells) H.B. Wang, M. Dembo, Y. Wang., *Am. J. Physiol. Cell Physiol.* **279**, C1345 (2000).
17. (Smooth muscle cells) A. Engler *et al. Biophys. J.* **86**, 617 (2004).
18. R. E. Mahaffy, C. K. Shih, F. C. MacKintosh, J. Kas, *Phys. Rev. Lett.* **85**, 880 (2000).
19. The Hertz model for a spherical probe of radius R indenting a thick slab gives $E \sim f/d^{3/2}R^{1/2}$ where the force f indents to a depth d. The shear modulus, G, of an isotropic, incompressible material is $G \approx E/3$, reflecting the fact that shearing and tensile extension are related by a rotation of frame.
20. C. M. Lo, H. B. Wang, M. Dembo, Y. Wang, *Biophys. J.* **79**, 144 (2000).
21. C. Storm, J. J. Pastore, F. C. MacKintosh, T. C. Lubensky, P. A. Janmey, *Nature* **435**, 191 (2005).
22. Y. C. Fung, *A First Course in Continuum Mechanics: For Physical and Biological Engineers and Scientists* (Prentice Hall, Englewood Cliffs, NJ, ed. 3, 1994).
23. M. M. Stevens, J. George, *Science* **310**, 1135 (2005).
24. A. Gefen, S. S. Margulies, *J. Biomech.* **37**, 1339 (2004).
25. S. Hu *et al.*, *Am. J. Physiol. Cell Physiol.* **287**, C1184 (2004).
26. G. Bao, S. Suresh, *Nat. Mater.* **2**, 715 (2003).
27. F. Wottawah *et al.*, *Phys. Rev. Lett.* **94**, 098103 (2005).
28. (Skeletal Muscle Cells) A. J. Engler *et al.*, *J. Cell Biol.* **166**, 877 (2004) .
29. Y. Yoshikawa, T. Yasuike, A. Yagi, T. Yamada, *Biochem. Biophys. Res. Commun.* **256**, 13 (1999).
30. S. Diridollou *et al.*, *Skin Res. Technol.* **6**, 214 (2000).
31. W. M. Leader, D. Stopak, A. K. Harris, *J. Cell Sci.* **64**, 1 (1983).
32. E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, *Science* **294**, 1708 (2001).
33. M. Chrzanowska-Wodnicka, K. Burridge, *J. Cell Biol.* **133**, 1403 (1996).
34. O. J. Pletjushkina *et al.*, *Cell Motil. Cytoskeleton* **48**, 235 (2001).
35. N. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7765 (2001).
36. P. C. Bridgman, S. Dave, C. F. Asnes, A. N. Tullio, R. S. Adelstein, *J. Neurosci.* **21**, 6159 (2001).
37. (Neurons) L. A. Flanagan, Y. E. Ju, B. Marg, M. Osterfield, P. A. Janmey, *Neuroreport* **13**, 2411 (2002).
38. M. A. Griffin, S. Sen, H. L. Sweeney, D. E. Discher, *J. Cell Sci.* **117**, 5855 (2004).
39. P. C. Georges, P. A. Janmey, in preparation.
40. T. Yeung *et al.*, *Cell Motil. Cytoskeleton* **60**, 24 (2005).
41. Y. Wang, in preparation.
42. P. C. Georges, P. A. Janmey, *J. Appl. Physiol.* **98**, 1547 (2005).
43. G. P. Raeber, M. P. Lutolf, J. A. Hubbell, *Biophys. J.* **89**, 1374 (2005).
44. A. Saez, A. Buguin, P. Silberzan, B. Ladoux, *Biophys J.*, in press .
45. A. Engler, L. Richert, J. Wong, C. Picart, D. E. Discher, *Surf. Sci.* **570**, 142 (2004).
46. Phagocytosis of rigid yeast particles by neutrophils involves a large contractile stress (~1 kPa) and implicates myosin (*47*), consistent with stiffness sensitivity of phagocytosis by macrophages (*48*).
47. E. A. Evans, A. Leung, D. Zhelev, *J. Cell Biol.* **122**, 1295 (1993).
48. (Macrophages) K. A. Beningo, Y. Wang, *J. Cell Sci.* **115**, 849 (2002).
49. M. Jin, I. Andricioaei, T. A. Springer, *Structure (Cambridge)* **12**, 2137 (2004).
50. A. F. Straight *et al.*, *Science* **299**, 1743 (2003).
51. B. Hinz, G. Celetta, J. J. Tomasek, G. Gabbiani, C. Chaponnier, *Mol. Biol. Cell* **12**, 2730 (2001).
52. A. Katsumi *et al.*, *J. Cell Biol.* **158**, 153 (2002).
53. (Smooth muscle cells) S. R. Peyton, A. J. Putnam, *J. Cell. Physiol.* **204**, 198 (2005).
54. N. Zaari, P. Rajagopalan, S. K. Kim, A. J. Engler, J. Y. Wong, *Adv. Mater.* **16**, 2133 (2004).
55. S. L. Goodman, G. Risse, K. von der Mark, *J. Cell Biol.* **109**, 799 (1989).
56. M. H. Zaman, R. D. Kamm, P. Matsudaira, D. A. Lauffenburger, *Biophys. J.* **89**, 1389 (2005).
57. P. Y. Jay, P. A. Pham, S. A. Wong, E. L. Elson, *J. Cell Sci.* **108**, 387 (1995).
58. D. Riveline *et al.*, *J. Cell Biol.* **153**, 1175 (2001).
59. L. P. Cramer, T. J. Mitchison, *J. Cell Biol.* **131**, 179 (1995).
60. A. Doyle, W. Marganski, J. Lee, *J. Cell Sci.* **117**, 2203 (2004).
61. M. Tamada, M. P. Sheetz, Y. Sawada, *Dev. Cell* **7**, 709 (2004).
62. I. Fillingham *et al.*, *Structure (Cambridge)* **13**, 65 (2005).
63. Recent unfolding of spectrin helical bundles at force levels that myosins can generate are described in (*64*).
64. R. Law *et al.*, *Biophys. J.* **85**, 3286 (2003).
65. Y. T. Shiu *et al.*, *Biophys. J.* **86**, 2558 (2004).
66. A. R. Asthagiri, C. A. Reinhart, A. F. Horwitz, D. A. Lauffenburger, *J. Cell Sci.* **113**, 4499 (2000).
67. T. Wakatsuki, R. B. Wysolmerski, E. L. Elson, *J. Cell Sci.* **116**, 1617 (2003).
68. A. Nicolas, B. Geiger, S. A. Safran, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12520 (2004).
69. I. B. Bischofs, U. S. Schwarz, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9274 (2003).
70. M. S. Steinberg, *Science* **137**, 762 (1962).
71. J. P. Trinkaus, J. P. Lentz, *Dev. Biol.* **89**, 115 (1964).
72. K. Jakab, A. Neagu, V. Mironov, R. R. Markwald, G. Forgacs, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2864 (2004).
73. R. A. Foty, M. S. Steinberg, *Dev. Biol.* **278**, 255 (2005).
74. S. E. Zalik, E. Lewandowski, Z. Kam, B. Geiger, *Biochem. Cell Biol.* **77**, 527 (1999).
75. E. A. Evans, V. Heinrich, F. Ludwig, W. Rawicz, *Biophys. J.* **85**, 2342 (2003).
76. N. K. Noren, W. T. Arthur, K. Burridge, *J. Biol. Chem.* **278**, 13615 (2003).
77. W. T. Arthur, N. K. Noren, K. Burridge, *Biol. Res.* **35**, 239 (2002).
78. In vitro studies of cadherin-coated surfaces show no cytoskeleton assembly and no response to contractile inhibitors (*79*).
79. H. Delanoe-Ayari, R. Al Kurdi, M. Vallade, D. Gulino-Debrac, D. Riveline, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2229 (2004).
80. A. K. Harris, *J. Theor. Biol.* **61**, 267 (1976).
81. A. Engler, S. Sen, H. L. Sweeney, D. E. Discher, in preparation.
82. R. G. Wells, *J. Clin. Gastroenterol.* **39**, S158 (2005).
83. M. A. Griffin *et al.*, *J. Cell Sci.* **118**, 1405 (2005).
84. E. J. Semler, P. A. Lancin, A. Dasgupta, P. V. Moghe, *Biotechnol. Bioeng.* **89**, 296 (2005).
85. H. J. Kong *et al.*, *Nat. Mater.* **4**, 460 (2005).
86. K. A. Beningo, M. Dembo, Y. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 18024 (2004).
87. G. Baneyx, L. Baugh, V. Vogel, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 5139 (2002).
88. A. L. Sieminski, R. P. Hebbel, K. J. Gooch, *Exp. Cell Res.* **297**, 574 (2004).
89. Y. Shikinami, M. Okuno, *Biomaterials* **24**, 3161 (2003).
90. P. F. Gratzer, J. P. Santerre, J. M. Lee, *Biomaterials.* **25**, 2081 (2004).
91. K. Rottner, A. Hall, J. V. Small, *Curr. Biol.* **9**, 640 (1999).

92. Support was provided by NIH, NSF-PECASE (D.E.D.), and Penn's NSF-MRSEC.

10.1126/science.1116995

REVIEW

Rigid Biological Systems as Models for Synthetic Composites

George Mayer

Advances that have been made in understanding the mechanisms underlying the mechanical behavior of a number of biological materials (namely mollusk shells and sponge spicules) are discussed here. Attempts at biomimicry of the structure of a nacreous layer of a mollusk shell have shown reasonable success. However, they have revealed additional issues that must be addressed if new synthetic composite materials that are based on natural systems are to be constructed. Some of the important advantages and limitations of copying from nature are also described here.

Rigid biological materials, such as shells, bone, and sponge spicules, have been attractive as models for synthetic structural composites because of their unusual combinations of mechanical properties, such as strength, stiffness, and toughness. A study by the National Materials Advisory Board (*1*) dealt with the broad area of biology as a guide for new materials technology, and that study has been followed, in recent years, by books and reports of symposia on biomimicry and bio-inspired materials [such as (*2*, *3*)]. The subject of bone and its structure and mechanics has been extensively treated in a comprehensive work by Currey (*4*), and much work has also been done on many other aspects of bone, such as the creation of both natural and synthetic bioresorbable scaffolding for repairs (*5*). The body of work on bone is extensive, and the subject of the mimicking of bone deserves a separate review. Therefore, the present work deals only with the mechanisms underlying toughening in mollusk shells and sponge spicules.

Increasing attention has been devoted, in the past three decades, to the mechanical behavior of the shells of mollusks. Currey was the first to describe the unusual toughness possessed by mother-of-pearl, the structure of nacre (*6*), and the wide diversity of structural morphologies that have been found in seashells (*7*) (Fig. 1).

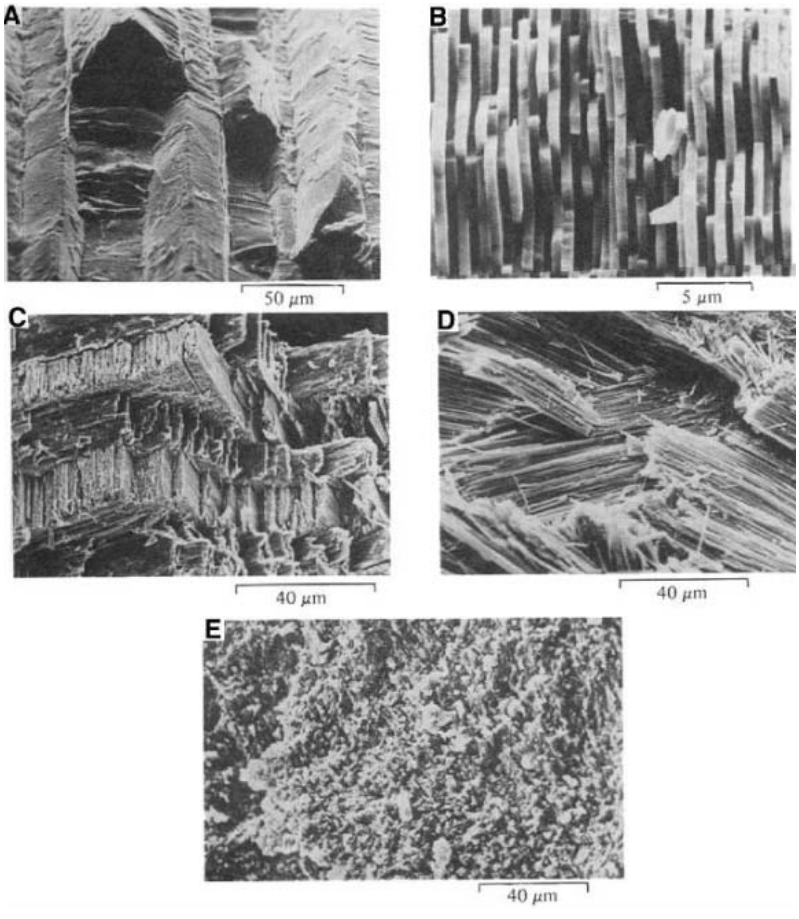


Fig. 1. Scanning electron micrographs, at various magnifications, of the fracture surfaces of various mollusk shell structures: (**A**) prismatic, (**B**) nacreous, (**C**) cross-lamellar, (**D**) foliated, and (**E**) homogeneous (*7*). [Used with permission of the Society for Experimental Biology]

A key work by Jackson, Vincent, and Turner (*8*) illustrated examples of the attractive combination of properties associated with nacre, in comparison with those of synthetic composites that had high volume percent (*v*/*o*) of ceramic phase, along with an organic minor phase as matrix. Two important features of nacre that distinguished it from the others in the study were the closely packed layered architecture and the soft matrix (or minor) phase. Nacre was also half as tough in the dry state as in the wet state (a factor that will be discussed in a subsequent section).

The attractive combinations of mechanical properties of many rigid biological materials stem from the fact that they are hybrid composites, consisting of a very small volume fraction of organic components (on the order of 1 to 5 *v*/*o*) surrounding a ceramic phase. The architecture of a nacreous structure is shown schematically in Fig. 2. Often, natural rigid materials that are found in the oceans have a large preponderance (on the order of 95 *v*/*o*) of a ceramic component, such as $CaCO_3$ (mollusk shells) or SiO_2 (spicules of sponges that live in cold waters), that has shown very limited toughness when used in its monolithic form.

Generally, what has been copied from nature for building synthetic structural composites has been the architectural configurations and the material characteristics rather than the specific natural materials that were originally found. This approach has limitations. A complicating and difficult issue has been the enormous potential problem of copying architectural features that are found in nature, at the micro and nano scales, into real, macroscale structural materials at reasonable cost.

Coincidentally, at least two of the structures shown in Fig. 1, the nacreous and crossed-lamellar, are seen, respectively, in the brick-and-mortar architecture of many buildings and in plywood

Department of Materials Science and Engineering, University of Washington, Roberts Hall 335, Box 352120, Seattle, WA 98195–2120, USA. E-mail: gmayer@u.washington.edu

(albeit at a much different scale and with different material components).

Mechanisms Underlying Resistance to Failure in Rigid Natural Materials: Mollusk Shells

In considering the toughness of rigid biological composites, an interpretation of toughness that is different from that used for conventional structural materials should be used. In the latter case, fracture toughness has to do with resistance to the propagation of cracks. This is generally measured using so-called *R* curves (measures of resistance to unstable crack propagation), under the assumption that crack propagation is fairly stable and linear. In the natural rigid composites that have been studied by myself and others, crack propagation is far from linear, and toughness in those materials should be reinterpreted as how much energy can be absorbed and dissipated before catastrophic failure. A convenient measure of energy dissipation may be estimated from the area under the load-deflection curve in the bending of a beam of the material. This has complex meaning for composites with high *v/o* of ceramic phase, because mechanisms that include various forms of fracture actually dissipate much energy. In addition, those mechanisms can be substantially assisted by the unusual properties of the thin, tenacious organic phases. The latter have been observed to elongate extensively both elastically and viscoelastically. Although many of the processes for energy dissipation in natural materials involve the creation of new surfaces, by no means is that the whole story. At least 10 mechanisms have been observed to contribute to energy dissipation in mollusk shell materials. These are:

1. Creation of new surface area by fracture and delamination; multiple microcracking is included here (*9*).
2. Crack diversion.
3. Pull-out of the ceramic phase from the minor organic component, perhaps aided by asperities (from mineral bridges) on the platelet surfaces, which provide frictional resistance against pull-out of the platelets (*10*).
4. Hole formation at the ends of the displaced ceramic-phase elements (which seems similar to stress-whitening in polymers) at larger deformations (*11*).
5. A high level of anchoring of the organic adhesive phase.
6. Ligament or filament formation in the organic phase, which is viscoelastic (*12*) as well as highly resilient.
7. Crack bridging by ligaments of the organic phase.
8. Unfolding of chains, breaking of cross links (*13*), and perhaps permanent reorientation of the organic phase during deformation.
9. Moisture has a substantial plasticizing effect on proteinaceous layers, thus leading to increases in the work required to cause fracturing (*8*, *14*).
10. Contributions of residual stresses to energy absorption (*15*). This was observed in a shell where two different adjacent structural forms were present in a multilayered structure.

How much energy could be dissipated by each of these mechanisms, and under what conditions (varying strain rates and temperatures, for example) they would be partitioned and triggered to operate, are presently unknown.

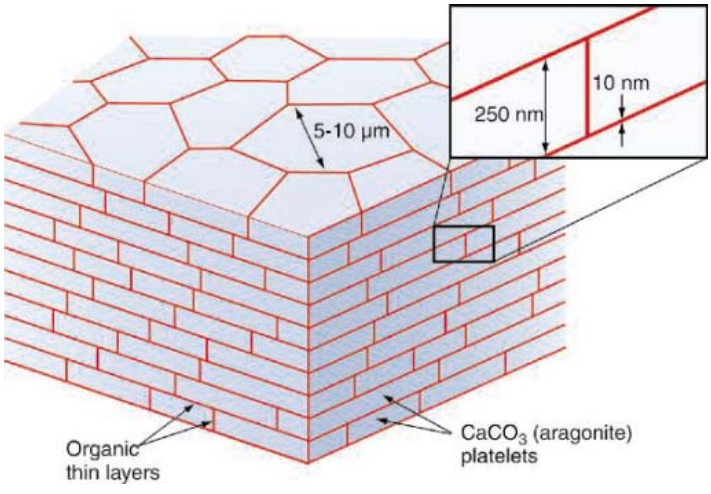


Fig. 2. Schematic diagram of nacreous structure. The organic thin film indicated between the layers also covers all other surfaces of each structural unit.

We have studied the mechanisms that control the attractive combinations of strength, stiffness, and energy absorption in a nacreous shell structure and have attempted to build synthetic composites at engineering scales (*16*). The nacre structure, found in the shell of the red abalone *Haliotis rufescens*, was our model. The important features of that structure showed (i) the existence of a space-filling, layered, and segmented architecture on the micro and nano scales. As shown schematically in Fig. 2, this is a "brick-and-mortar" structure, with the bricks approximating hexagonal and other multisided platelets (from the top view). (ii) The major constituent is a ceramic phase, $CaCO_3$, of a high volume fraction, along with a thin, viscoelastic, and resilient organic constituent (consisting of proteins and possibly other organic materials). The thin layer of the minor constituent is termed the matrix; it encases the ceramic component on all sides and shows great adherence to the ceramic phase.

Development of a Biomimetic Synthetic Composite

We designed simplified synthetic segmented composite beams, based on the brick-and-mortar structure shown in Fig. 2. Materials were selected, and composites were built on the macro scale. Although nacre has micro- and nanoscale features, macroscale structures were justified by observations of the mechanical behavior of a synthetic ceramic/organic segmented material that had been proposed for use as armor and reported in (*16*). The stacking architecture of the segmented composite plates had also been studied (*17*).

In this effort, there were a number of interesting sidelights, which illustrate the difficulty of mimicking natural composites. The first of these was that ceramic materials such as $CaCO_3$ and SiO_2 are not normally considered for components of structural composites, because their mechanical properties are generally insufficient for such purposes. Therefore, Al_2O_3 (or alumina), a "workhorse" structural ceramic, was chosen as the ceramic component for the synthetic composite, which was patterned after a simplified nacre architecture. The organic component of nacre appeared to have the characteristics of a good adhesive. However, when a very strong adhesive, such as a silicone-based material, was used and beams of alumina/silicone adhesive were built (in segmented fashion) for bend tests, cracks were found to traverse across the thickness of the beam quite easily. What had actually been observed in the failure of nacre was that the organic (adhesive) phase allowed for reasonable strength but also would delaminate and promote crack diversion, while exhibiting fibril formation during large deformation as well as strong tenacity to the ceramic substrate. The search for a suitable synthetic adhesive with nacre-like behavior was assisted by advice from a leading commercial adhesives source (*18*).

A second problem was that conventional monolithic ceramics generally need to have a very smooth surface finish for good fracture strength. On the other hand, a polished surface is normally not helpful for the bonding of an adhesive.

One of the important findings of these experiments was that there appeared to be a maximum critical level of the organic phase that controlled energy dissipation. Exceeding

that level meant that energy dissipation decreased (Fig. 3). Also, although layered ceramic composites with continuous layers showed greater strength and stiffness, energy dissipation was not as high as that shown by the segmented composite with low organic adhesive content.

The important role of very thin layers in controlling the energy dissipation in natural ceramic/organic composites has been a key finding. When the amount of the adhesive constituent is at a critical level, it appears that a multitude of mechanisms of energy dissipation are triggered. On the other hand, when that *v/o* of adhesive is larger than a critical volume fraction, the available modes seem to be much more limited. It remains to be seen whether a smaller amount of adhesive component in the composite would yield even greater levels of energy dissipation. Also, it has not been determined how energy is distributed and dissipated among the various mechanisms, such as the creation of new surfaces, crack bridging, ligament formation, unfolding of molecular chains, chain scission, etc.

It should also be noted that, normally, in the consideration of energy absorption by a structure, the elastic contribution is recovered and given back to the loading frame of the testing machine. In the case of these inorganic/organic systems, it is not yet known how much energy is elastic and how much is viscoelastic. However, in Fig. 3, the extended range of the synthetic composite with the low *v/o* of adhesive phase covers a much larger zone than do the other examples that were tested. It was therefore concluded that the energy dissipation in the former was much greater than that shown by the latter composites.

During the past several years, several investigators (*19*, *20*) have claimed that continuous structural laminates of certain ceramic/metallic or intermetallic/metallic composites were biomimetic in their origins and were related to the superior combinations of mechanical properties that have been shown by nacre. Although continuous laminated composites of those materials do exhibit attractive mechanical properties (and showed retardation of crack propagation), they do not closely mimic the structures of mollusks. There are several reasons why this is so: (i) There is no report of ligament formation in the ductile metallic layers of such composites, and that may be one of the key energy-absorbing mechanisms underlying energy dissipation in nacreous structures. (ii) There has been no sign of crack bridging by the metallic component. (iii) The layers in materials such as nacre are segmented, rather than continuous. (iv) There has been no reported indication of very large resilience in these synthetic continuous composites, as is shown in natural rigid systems.

Mechanisms of Mechanical Behavior of Siliceous Sponge Structures

Another class of interesting natural structural composites can be found in the spicules of Hexactinellid sponges. Spicules are building components of the supports and skeletons of many sponges. They can be either calcareous or siliceous. Observations reported by Levi *et al.* (*21*) on silica-based spicules of a *Monorhaphis* sponge generated great interest because of their combination of properties, namely toughness (the new definition as energy dissipation applies here, also) combined with stiffness, and resilience. A pencil-sized rod spicule, on the order of a meter in length, could be bent into a circle without breaking. When the load was released, the spicule recovered its original shape. When the bending of the spicule rod was compared with that of a silica rod, the toughness of the spicule was found to be nearly an order of magnitude higher. What differentiated the structure of the spicule rod from that of the silica rod was the presence of concentric rings that were separated by very thin organic layers. The structure of a similar but smaller sponge spicule is shown in Fig. 4. Layers of hydrated silica were found to be separated by much thinner organic layers. In the central core of the spicules (not shown in Fig. 4) is a square cross-section of protein filament, about 1 μm on each side.

Similar to the case of nacre, thin, flexible, tenacious layers have been proposed as a major underlying reason for the large energy dissipation and resilience of the spicules (*22*). When fractured spicules were examined, the layers were found to be quite effective diverters of cracks. The similarity of Hexactinellid spicules to nacre seems to extend to the presence of very thin layers of complex organic material, in this case, well-bonded to a hydrated (silicate glass) substrate. We have estimated the volume fraction of organic constituents, including the central core region, in the spicules of *Euplectella aspergillum* to be on the order of 2 to 3 *v/o* (*23*). However, in the case of spicules, the cylindrical components appear to be continuous rather than segmented along their lengths. The mechanisms that contribute to the ability to absorb energy are expected to be similar to those that have been observed in nacreous structures, but do not include the mechanisms that are related to segmented structures, such as the pull-out of platelets.

The central protein filaments of the spicules of a different species of siliceous sponge (not layered) have been studied by Morse and colleagues (*24*) and termed silicateins (for silica proteins). Three different silicatein subunits have been characterized. These are thought to control the growth and form of the subsequent silica deposition. The central filament of *E. aspergillum* remains to be characterized and will likely yield results different from those of prior studies.

The initial observations about toughening in the study by Levi *et al.* were borne out by studies on other Hexactinellid sponge species (*22*, *25*). We have examined the behavior of spicules of *E. aspergillum* in bending and under tension and have found them to be much tougher [that is, they dissipated much more (six to seven times more) energy during deformation until final failure] than conventional glass fibers of similar sizes (*23*). The skeleton of this sponge also appears to be a torsion-resistant structure, as discussed in the recent comprehensive paper on the structural hierarchy in this system by Aizenberg *et al.* (*26*). Structure indeed appears to follow function here. In fact, the weave that is found in the *E. aspergillum* skeleton (without the helical collar of reinforcements) is similar to glass windings such as those found in modern woven glass/epoxy composites. At this point in time, no known attempts have been made to copy these siliceous structures.

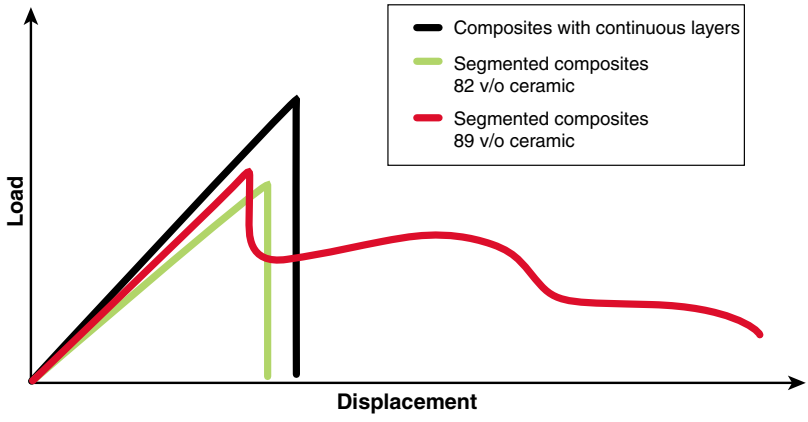


Fig. 3. Schematic bend test results of laminated synthetic composites (*17*); the energy dissipated is estimated from the area under each curve.

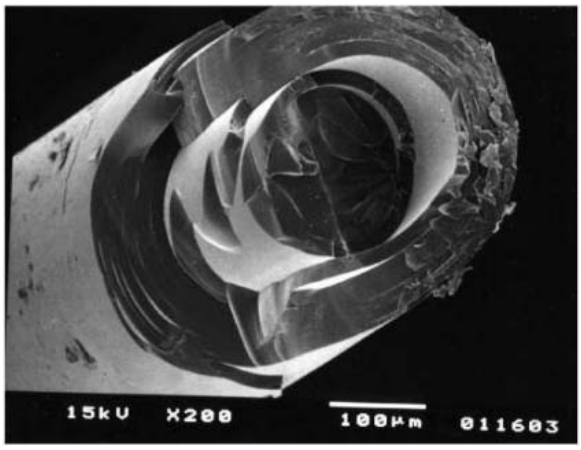


Fig. 4. Scanning electron micrograph of fractured Hexactinellid sponge spicule, showing concentric ring structure (*32*).

Advantages and Disadvantages of Copying from Nature

The first issue that inevitably arises in looking at natural materials and structures is the question "are the systems optimized?" and the answer is probably "yes and no." No, because of the limitations in the choice of elements (C, Ca, P, Si, H, O, etc.) and ions that are found in the particular natural environment. We know that, for engineering purposes, we are able to select and use stronger and more durable materials for structures, and we may need them to function over much wider temperature regimes. On the other hand, we should recognize that natural structures are designed to survive in environments that have restricted mechanical loads, fairly narrow temperature regimes, and so on. The living plant or animal that cannot adapt its structure and properties to those environments or to changes in them does not survive. If environments change gradually over time, some biological structures may be able to adapt. In the search for survival, living systems such as mollusks and sponges have used sophisticated biomineralization mechanisms that provide the organisms with hybrid structures that exhibit attractive combinations of strength, stiffness, resilience, and energy-absorbing capabilities (*27*). Thus, the synthesis of elegant structures and unique interfaces occurs under the relatively mild conditions (and with the right catalysts) that are found in the ocean, rather than under the much more harsh high-temperature processing that is normally done, for example, to make glass. It should, however, be emphasized that the proteins that exist in siliceous structures in the ocean would not survive processing at the high temperatures that are generally used to form glass.

The foregoing facts caution us not to expect that the structures found in the oceans would survive in more extreme environments of temperature and under other conditions that are outside of their operating envelopes. Of importance for creating rigid structural materials, thus far, in the area of biomimicry, are two major findings. The first is applying architectural lessons in building new hybrid composites [this has also been noted recently by Currey (*28*)]. From the work reported in (*16*), it appears that architecture is a governing factor, as well, in building new materials at different length scales. The second important finding is that a number of mechanical properties (such as ductility, resilience, and the ability to dissipate energy) are controlled by the thin organic layers in a rigid natural material such as nacre. These layers (including those found in sponges) are much more complex than the synthetic adhesives that have been used in the building of segmented composite beams.

Some years ago, Weiner, Traub, and Parker proposed a model of the various constituents in the thin layers surrounding nacre platelets (*29*), and researchers at the University of California at Santa Barbara have characterized an organic adhesive fibrillar component that is responsible for the large extension in nacre (*30*). In *H. rufescens*, three protein components have been identified and connected with various roles in biomineralization. More recent work in Bremen (*31*) has clarified the key role of the nacre protein perlucin in nucleating the growth of calcium carbonate crystals in the marine snail *H. laevigata*. Thus, serious complexities and connections between the biomineralization, mechanical behavior, and mechanics of the organic constituents in natural rigid composites remain to be addressed and solved.

Other important factors that affect the mechanical properties of rigid biological materials are that these materials are generally highly directional and also are beneficially affected by moisture. Moisture contributes greatly to the dissipation of energy, through plasticization of the small volume of proteinaceous matrix.

The organic phases in these rigid natural composite materials, whether calcium or silicon-based, also show viscoelasticity. This can be seen in the behavior of samples of nacre that were subjected to bending but had not been taken to failure (*16*), from strain-rate sensitivity tests on spicules of *E. aspergillum* (*22*), and from the work of Ji and Gao (*12*).

Of immense significance, too, are features that have been observed, but researchers have thus far been unable to replicate in synthetic systems, such as the ability for self-repair and the exceptional tenacity at interfaces.

References and Notes

1. *Hierarchical Structures in Biology as a Guide for New Materials Technology, NMAB-464* (National Materials Advisory Board, National Academy Press, Washington, DC, 1990).
2. *Design and Nature,* C. A. Brebbia, L. J. Sucharov, P. Pascolo, Eds. (WIT Press, Southampton, UK, 2002).
3. *Biological and Bioinspired Materials and Devices*, vol. 823 of *Materials Research Society Symposium Proceedings*, J. Aizenberg *et al.*, Eds. (Materials Research Society, Warrendale, PA, 2004).
4. J. D. Currey, *Bones: Structure and Mechanics* (Princeton Univ. Press, Princeton, NJ, 2002).
5. Y. Zhang, M. Ni, M. Zhang, B. Ratner, *Tissue Eng.* **9**, 337 (2003).
6. J. D. Currey, *Proc. R. Soc. London Ser. B* **196**, 443 (1977).
7. J. D. Currey, in *Symposium XXX1V, The Mechanical Properties of Biological Materials* (Symposia of the Society for Experimental Biology, Cambridge Univ. Press, Cambridge, 1980), pp. 75–97.
8. A. P. Jackson, J. F. V. Vincent, R. M. Turner, *J. Mater. Sci.* **25**, 3173 (1991).
9. S. Kamat, X. Su, R. Ballarini, A. H. Heuer, *Nature* **405**, 1036 (2000).
10. A. G. Evans, Z. Suo, R. Z. Wang, M. Y. He, J. W. Hutchinson, *J. Mater. Res.* **16**, 2475 (2001).
11. R. Z. Wang, Z. Suo, A. G. Evans, N. Yao, I. A. Aksay, *J. Mater. Res.* **16**, 2485 (2001).
12. B. Ji, H. Gao, *J. Mech. Phys. Sol.* **52**, 1963 (2004).
13. Z. Tang, N. A. Kotov, S. Magonov, B. Ozturk, *Nat. Mater.* **2**, 413 (2003).
14. N. M. Neves, J. F. Mano, *Mater. Sci. Eng. C* **25**, 113 (2005).
15. D. J. Scurr, S. J. Eichhorn, in *Mechanical Properties of Bioinspired and Biological Materials*, vol. 844 of *Materials Research Society Symposium Proceedings*, C. Viney *et al.*, Eds. (Materials Research Society, Warrendale, PA, 2005), pp. 87–92.
16. G. Mayer, *Mat. Sci. Eng. C,* in press.
17. B. M. Gruner, thesis, University of Washington, Seattle, WA (2003).
18. A. V. Pocius, 3M Company, personal communication (March 2002).
19. M. Sarikaya *et al.*, in *Proceedings of the American Society for Composites, 5th Technical Conference* (Technomic Press, Lancaster, PA, 1990), pp. 176–182.
20. K. S. Vecchio, *J. Miner. Met. Mat. Soc.* **57**, 25 (2005).
21. C. Levi *et al.*, *J. Mater. Sci. Lett.* **8**, 337 (1989).
22. G. Mayer *et al.*, in *Mechanical Properties of Bioinspired and Biological Materials*, vol. 844 of *Materials Research Society Symposium Proceedings*, C. Viney *et al.*, Eds. (Materials Research Society, Warrendale, PA, 2005), pp. 79–86.
23. S. G. Walter, B. D. Flinn, G. Mayer, *Mat. Sci. Eng. C,* in press.
24. J. N. Cha *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 361 (1999).
25. M. Sarikaya *et al.*, *J. Mater. Res.* **16**, 1420 (2001).
26. J. Aizenberg *et al.*, *Science* **309**, 275 (2005).
27. S. Mann, *Biomineralization* (Oxford Univ. Press, Oxford, 2001).
28. J. D. Currey, *Science* **309**, 253 (2005).
29. S. Weiner, W. Traub, S. B. Parker, *Philos. Trans. R. Soc. London Ser. B* **304**, 425 (1984).
30. B. L. Smith *et al.*, *Nature* **399**, 761 (1999).
31. S. Blank *et al.*, *J. Microsc.* **212**, 280 (2003).
32. M. Rodriguez, thesis, University of Washington, Seattle, WA (2004).

33. I wish to express my appreciation to my present and former students who have helped enormously to study, identify, and elucidate the micromechanisms underlying mechanical behavior in rigid natural systems. Also, my thanks go to E. Baer and A. Hiltner, who brought fresh new insight into hierarchical structures in biological systems; to the late Sid Diamond of the U.S. Department of Energy, who strongly encouraged and supported the ongoing search for tough ceramic materials; and to my wife, Jane, for her help and patience.

10.1126/science.1116994

REVIEW

Inspirations from Biological Optics for Advanced Photonic Systems

Luke P. Lee* and Robert Szema

Observing systems in nature has inspired humans to create technological tools that allow us to better understand and imitate biology. Biomimetics, in particular, owes much of its current development to advances in materials science and creative optical system designs. New investigational tools, such as those for microscopic imaging and chemical analyses, have added to our understanding of biological optics. Biologically inspired optical science has become the emerging topic among researchers and scientists. This is in part due to the availability of polymers with customizable optical properties and the ability to rapidly fabricate complex designs using soft lithography and three-dimensional microscale processing techniques.

Whether it is to search for nourishment, evade predators, or seek protection from the elements, unique biological optical systems are tailored for the individual needs of each organism. Among these, the mechanism of sight is perhaps the most varied in the animal kingdom. Eyes for different species are optimized for day or night vision, for near or far, for wide or narrow fields of view, and so on (*1*).

Biologically inspired optical science is a relatively new and expanding field. The recent development of reconfigurable soft lithography (*2*) using polydimethylsiloxane (PDMS) allows the creation of unconventional three-dimensional (3D) polymeric optical systems similar to biological ones, which are themselves constructed from biological polymers. Studies in developmental biology and molecular biology are examining how protein crystals form lenses and why biomolecular actuations are needed for the control of different optical systems. Man-made biomimetic systems can be grouped according to the biological designs they attempt to emulate, including camera-type eyes, compound eyes, and others.

Eyes

The earliest scientific postulations of vision appeared during the time of the ancient Greeks, who described sight as elemental fire emanating from the eye (*3*). It wasn't until some fifteen hundred years later that al-Haytham accurately described how lenses can focus and magnify images (*4*), and the first accurate description of the human eye followed in 1604. Scientific fascination with eyesight has continued to the present day.

In its simplest form, sight requires that light rays be focused onto a light detector. The development of visual systems has been both convergent and progressive, with many species having accomplished similar functional goals through different paths. No fewer than 10 generalized optical mechanisms have been found in nature, each with its own variations (*5*). Two of the most prevalent are the camera-type eye and the compound eye.

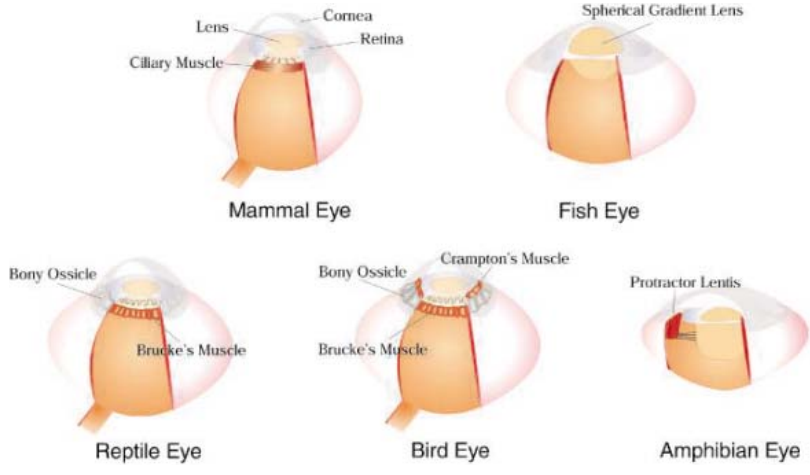


Fig. 1. Various types of camera-type eyes. (**A**) The arrangement of the mammalian ciliary muscle allows for passive changes in lens thickness. (**B**) The fish eye has a spherical gradient lens. (**C**) Brucke's muscles attached to bony ossicles in reptiles and birds, in contrast to the mammal and fish eyes, actively change the lens thickness. (**D**) Birds have an additional muscle, Crampton's muscle, which can alter the shape of the cornea. (**E**) The protractor lentis in some amphibian eyes moves a fixed-shape lens closer or farther from the retina for accommodation.

Camera-Eye Systems

The human eye is the most familiar biological optical system. It is categorized as a camera-type eye (Fig. 1A), which generally relies on a single lens to focus images onto a retina. In the human eye, focusing at different distances is made possible by a flexible and controllable crystalline lens. The ciliary muscles alter tension on the lens, changing its curvature and therefore its focal length.

In the animal kingdom, there are diverse types of camera eyes. For example, fish eyes have a spherical gradient index lens (Fig. 1B). The bird eye has the added control of reshaping and deforming the cornea as well (*5*). Brucke's muscles attached to bony ossicles in reptiles and birds actively change the lens thickness (Fig. 1C). Birds have an additional muscle, Crampton's muscle, which can alter the shape of the cornea (Fig. 1D). In contrast, the whale eye uses hydraulics to move the lens itself closer or farther from the retina; a chamber behind the lens is filled or emptied with fluid depending on the focal length needed (*6*). This design allows for good vision in and out of the water, and compensates for increased pressure in deeper aquatic environments. The protractor lentis in some amphibian eyes moves a fixed-shape lens closer or farther from the retina for accommodation (Fig. 1E).

Using soft lithography techniques, which allow for the creation of unconventional 3D polymer structures, a few groups have used these principles in designing an adaptive fluidic lens (*7*–*9*). However, these designs use a homogeneous spherical lens which suffers because the peripheral light rays are more refracted than the axial ones, leading to a type of astigmatism.

In cephalopods such as octopi and squid, this spherical aberration is solved by a ball-shaped lens with a spherically symmetric refractive index gradient that decreases from the center outward. This arrangement is particularly well suited for a watery environment, where the cornea does not provide an appreciably refractory change. That is, both sides of the cornea consist of a watery medium, and the entire focusing power of the

Berkeley Sensor and Actuator Center, Department of Bioengineering, University of California, Berkeley, CA 94720–1762, USA.

*To whom correspondence should be addressed. E-mail: lplee@berkeley.edu

eye is within the lens itself. This mechanism also provides the shortest possible focal length, while allowing for a wide field of view in a compact form.

The necessary refractive gradient of this natural lens was first postulated in the 1800s (*1*), a precise mathematical description was achieved in 1944 (*10*), and a man-made equivalent was constructed in 1986 (*11*). However, creating a biologically faithful retina is equally challenging. The retina of the cephalopod, and in many animals, is a curved structure. This is noteworthy because conventional electronic processing techniques to create photosensor arrays (as in cameras) are planar. To remedy this, Hung *et al*. have fabricated photodetectors with flexible interconnects, allowing for a curved artificial retina (*12*).

Compound Eyes

Although commonplace, the insect compound eye has an allure that stems in part from it being so different than our own. Its complexity is notable at first glance, with up to 10 thousand lenslets in some species of dragonflies (Fig. 2A). Grossly, compound eyes are divided into two types, superposition and apposition. As we have come to expect, each type is well adapted to the needs of its owner.

In the apposition compound eye, the individual facets are optically isolated from one another, with each providing part of the total scene (Fig. 2B). This is analogous to having individual cameras arranged spherically. From an engineering perspective, the apposition eye has its advantage in that images are processed in parallel, with each facet sending signals simultaneously. This allows for fast motion detection and image recognition. The trade-off, however, is that the brightness of the image is substantially diminished, as each facet can only capture a small amount of light.

Replicating an apposition compound eye does not lend itself well to efficiency or compactness, as each optical unit requires its own image capture and processing module. Still, the design concept is a relatively simple one, and new micromachining technologies have miniaturized these devices to a scale never before possible. Ogata *et al*. fabricated an artificial compound eye and integrated retina in the 1990s using planar arrays of gradient refractive index (GRIN) rods to focus light through pinholes onto a photodetector array (*13*) (Fig. 2D). Their resolution was only 16 by 16 but has led to modern versions that have the ability to capture full color images (*14*, *15*).

Even newer examples of apposition eyes have ommatidia arranged normal to a sphere, more faithful to their natural counterparts. The first artificial ommatidia by self-aligned microlenses and waveguides were created by Kim *et al*. (*16*). This was followed by a 3D compound eye with self-aligned waveguides and individual microlens units on a spherical surface by Jeong *et al*. (*17*). The ommatidia are arranged along a hemispherical polymer dome such that each points to a different direction, allowing for a wide field of view, similar to that of the natural eye (Fig. 2E). The spherical configuration of the microlenses was accomplished by a polymer replication process with the use of the deformed elastomer membrane, which has microlens patterns. The formation of self-aligned polymer cones and waveguides with respect to microlenses on the hemispherical dome was also realized by a self-writing process in a photosensitive polymer resin (Fig. 2F).

In the superposition compound eye, the images from each facet are not optically isolated. That is, they are projected in overlapping fashion onto a common retina (Fig. 2C). This increases photosensitivity, but may lead to blurring at the image interfaces. In biomimetics, this type of compound eye is not as popular as the apposition type, but it does have unique applications.

Historically, one of the most famous examples of biomimetic optics was described in 1979, when Angel proposed that lobster eyes be used as x-ray telescopes (*18*–*20*). Lobster eyes are composed of an array of tapered tubes which bend light by reflection rather than refraction (Fig. 2F). The light follows a path as if striking a spherical mirror. The tubes also share a common retina, making a superposition compound eye.

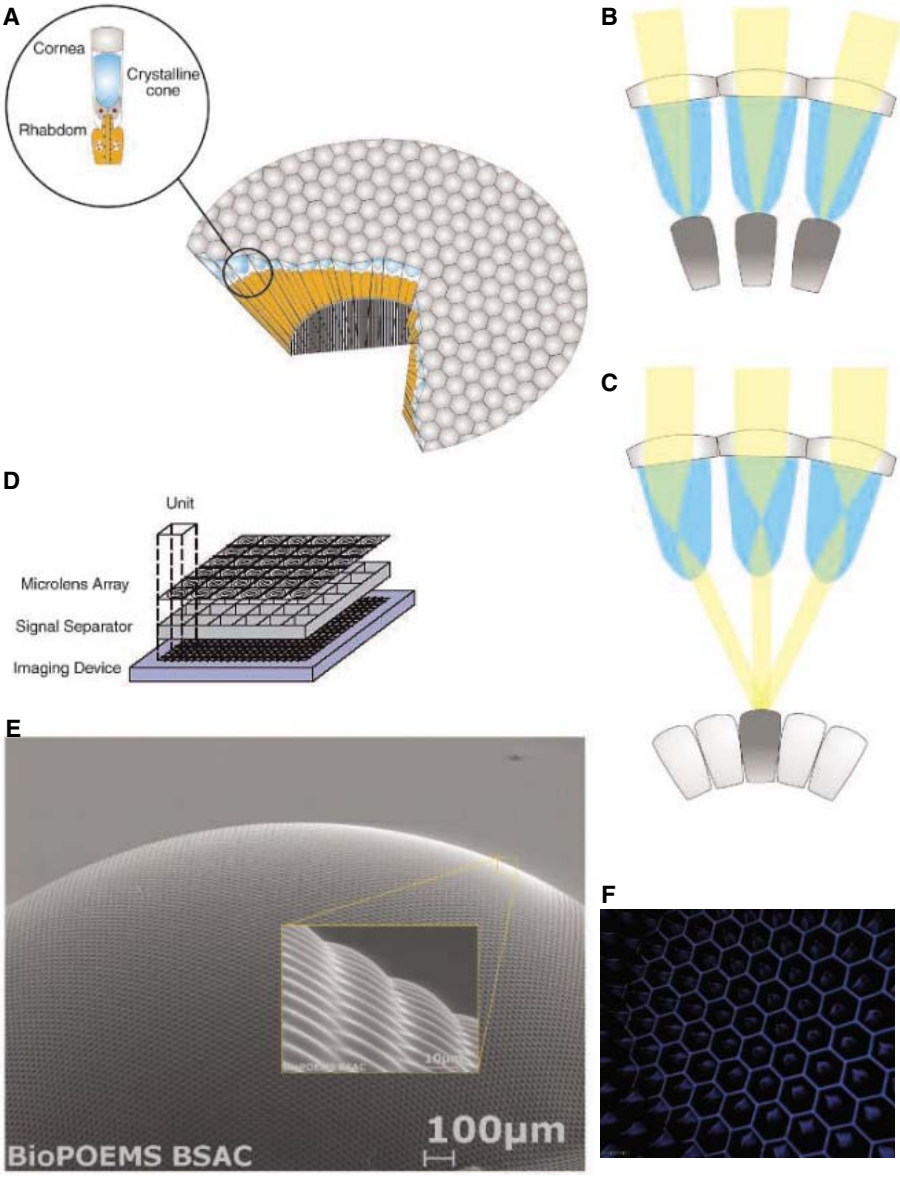


Fig. 2. Compound eye. (**A**) Schematics of compound eye. (**B**) Light flow through an apposition compound eye. (**C**) Light flow through a superposition compound eye. (**D**) Microlens array. [Image from (*13*)] (**E**) SEM image of an artificial compound eye fabricated by the biologically inspired 3D optical synthesis method. [Image from (*17*)] (**F**) Confocal micrograph of self-written cones under honeycomb-packed microlens array in the artificial compound eye. [Image from (*17*)]

Traditional x-ray telescopes use concave mirrors to reflect x rays, but only a small percentage of rays are bent in this manner. X rays are only reflected at glancing angles, leaving a 1° field of view. Arranged spherically, a lobster eye has the potential for an unlimited field of view. Another use for the control of x rays is to invert the lobster eye such that parallel beams can be produced from a point source (*18*–*20*).

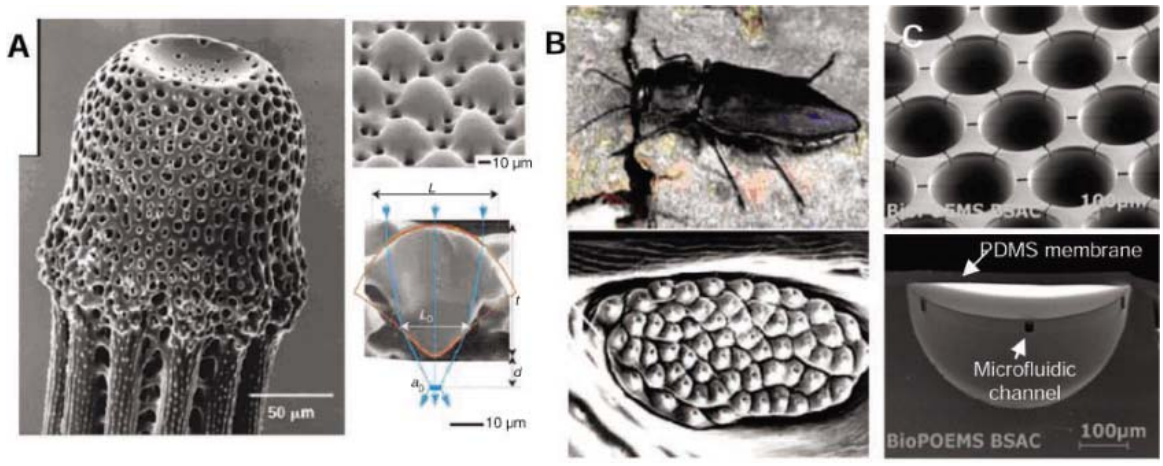


Fig. 3. Other biomimetic approaches. (**A**) SEM images of the brittlestar, indicating the focus of light rays at photosensitive locations beneath the surface. [Image from (*22*)] (**B**) An *M. acuminate* beetle with electron micrograph of its pit organ. [Image from (*24*)] (**C**) SEM images of biologically inspired microfluidic doublet lenses. (Top) Lens without thin PDMS elastomer. (Bottom) Cross-sectional image of the doublet lens with the thin PDMS elastomer membrane (*9*).

Finally, a hybrid apposition/superposition eye is under development by Szema *et al*. (*21*). Although its current form is not found in nature, this design draws its inspiration heavily from the biological world. Essentially, it involves a superposition eye with optical shutters attached to each of the facets of the eye. By opening one shutter at a time, images are retrieved from each facet individually, optically isolating each facet as in an apposition arrangement. However, these facets all project onto a common retina, mimicking the superposition compound eye. The advantages of this system are reduced processing requirements while maintaining separate, unblurred, images from each facet. The latter is particularly relevant given that the separated images can be used to determine distance to an object, much like humans use two eyes to perceive depth.

Other Systems in Nature

Commonly known as the brittlestar, the species *Ophiocoma wendtii* is able to evade predators and is sensitive to light, without obvious eyes or a brain. In 2001, Aizenberg *et al*. discovered that the calcite crystals throughout the brittlestar skeletal body were parts of an all-encompassing compound eye (*22*) (Fig. 3A). The crystals (40 to 50 μm in diameter) form doublet lenses which correct spherical aberration and birefringence. The focal point of the lenses matches nerve bundle locations beneath the crystal array. Commercial applications of such crystals have applications in optical networking and improved photolithography methods. They have been able to produce single calcite crystals with a defined crystallographic orientation by using micropatterned templates (*23*).

Jeong *et al*. have used this same design in a microfluidic doublet lens system capable of creating dual modes (*9*) (Fig. 3C). It can be tuned either by changing the shape of a fluidic lens or by changing the refractive index of the filling media. In this way, the system is able to minimize optical aberrations while maximizing the range of focal length or field of view.

The beetle *Melanophila acuminate* is another curious creature with the unique ability to detect forest fires some 80 km away. Female beetles lay eggs in burnt trees which no longer maintain their natural defenses. This is the only environment in which their larvae survive. They are directed to fires by specialized pit organs which are tuned to a specific infrared frequency (Fig. 3B). These organs hold 50 to 100 sensors, each 15 μm in diameter, which absorb the infrared light. Expansion of a cuticular apparatus is detected by mechanoreceptors which, in turn, direct the beetle (*24*).

Researchers are developing and characterizing new materials that behave similarly in response to heat (*25*). The expansion of these materials in response to various parts of the infrared spectrum allow for the detection of specific sources.

The Future

Imitating nature is a complex endeavor, and a blind biomimetic approach is not the best methodology. Instead, molecular-level studies of the biological development of natural vision systems are key. For example, current infrared sensors can distinguish more than what human eyes can see, but they require a sophisticated cooling system to work. Somehow, insects have this same ability without the limitation of temperature control. This is but one example of how it is primarily nature's designs that are superior to man-made equivalents. However, if we are able to decode the designs, then the combination of our creativity in materials and nature's wisdom is synergistic one with incredible potential.

References and Notes

1. M. F. Land, *Contemp. Phys.* **29**, 435 (1988).
2. Y. Xia *et al.*, *Science* **273**, 347 (1996).
3. C. Millerd, *On the Interpretation of Empedocles (Ancient Philosophy)* (Garland, New York, 1980).
4. A. Sabra, *The Optics of Ibn-Haytham: On Direct Vision (Studies of the Warburg Institute)* (The Warburg Institute, London, 1989).
5. M. Land, D. Nilsson, *Animal Eyes* (Oxford Univ. Press, Oxford, 2002).
6. R. Smythe, *Vision in the Animal World* (Macmillan Press, London, 1975).
7. D. Zhang, V. Lien, Y. Berdichevsky, J. Choi, Y. Lo, *Appl. Phys. Lett.* **82**, 3171 (2003).
8. N. Chronis, K. Jeong, G. L. Liu, L. P. Lee, *Opt. Express* **11**, 2370 (2003).
9. K. Jeong, G. L. Liu, N. Chronis, L. P. Lee, *Opt. Express* **12**, 2494 (2004).
10. R. K. Luneberg, in *Mathematical Theory of Optics* (Brown Univ. Press, Providence, RI, 1944), pp. 208–213.
11. Y. Koike, Y. Sumi, Y. Ohtsuka, *Appl. Opt.* **25**, 3356 (1986).
12. P. Hung, K. Jeong, G. Liu, L. P. Lee, *Appl. Phys. Lett.* **85**, 6051 (2004).
13. S. Ogata, J. Ishida, T. Sasano, *Opt. Eng.* **33**, 3649 (1994).
14. J. Tanida *et al.*, *Opt. Express* **11**, 2109 (2003).
15. J. Duparre *et al.*, *Proc. SPIE* **5346**, 89 (2004).
16. J. Kim, K. Jeong, L. P. Lee, *Opt. Lett.* **30**, 5 (2005).
17. K. Jeong, J. Kim, J., L. P. Lee, "Biologically inspired optical systems" (available at http://biopoems.berkeley.edu).
18. J. Angel, *Astrophys. J.* **233**, 364 (1979).
19. B. K. Hartline, *Science* **207**, 47 (1980).
20. M. Denton, *Nature's Destiny* (The Free Press, New York, 1998), p. 354.
21. R. Szema, J. Rastegar, L. P. Lee, *J. Med. Eng. Technol.* **28**, 117 (2004).
22. J. Aizenberg, A. Tkachenko, S. Weiner, L. Addadi, G. Hendler, *Nature* **412**, 819 (2001).
23. J. Aizenberg, D. Muller, J. Grazul, D. Hamann, *Science* **299**, 1205 (2003).
24. L. Sowards, H. Schmitz, D. Tomlin, R. Naik, M. Stone, *Ann. Entomol. Soc. Am.* **94**, 686 (2001).
25. J. Roach, "U.S. military looks to beetles for new sensors," *National Geographic News*, 8 March 2004 (available at http://news.nationalgeographic.com/news/2003/03/0314_030314_secretweapons3.html).
26. We thank L. J. Buckley for his support and guidance. This work was supported by the Defense Science Office of Defense Advanced Research Project Agency.

10.1126/science.1115248

Directionally Controlled Fluorescence Emission in Butterflies

Pete Vukusic* and Ian Hooper

In synthetic optical emitters such as light-emitting diodes (LEDs), the majority of generated light is trapped internally. Recently developed high-efficiency devices, however, use two-dimensional (2D) photonic-crystal geometries to enhance the extraction efficiency of light, and the devices also use distributed Bragg reflectors (DBRs) to control emission direction. Here we detail the elaborate optical emission system on the wing scales of a small group of butterflies. Their scales comprise a pigment-infused 2D photonic crystal that provides intense directed fluorescence, which is directionally enhanced by a DBR. This biological system shares many design features with high-emission LEDs.

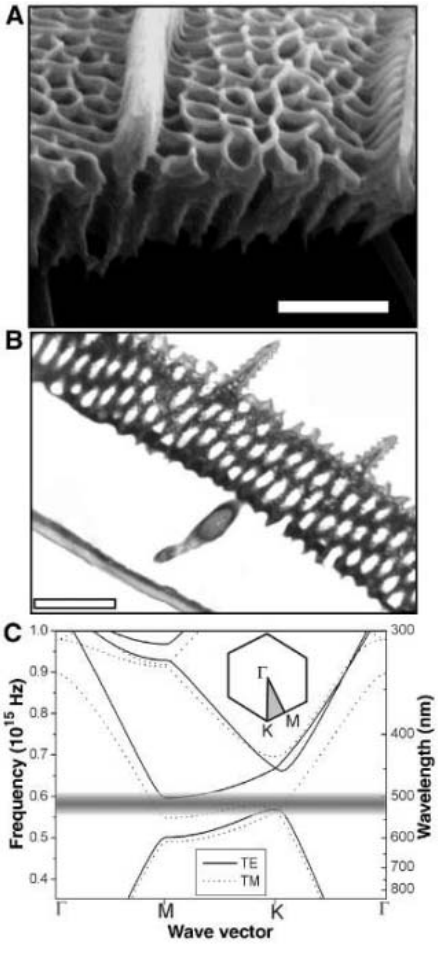


Fig. 1. (**A**) Scanning electron microscope image of the PCS in the *P. nireus* colored scale showing fractured air cylinder edges. Scale bar, 1 μm. (**B**) Transmission electron microscope image of a section through a *P. nireus* colored scale, taken at a small angle to the plane of the PCS. Scale bar, 1 μm. (**C**) Band diagram of *P. nireus* intra-domain PCS structure (the horizontal bar at 505 nm represents fluorescence emission full width at half maximum).

Swallowtail (*Papilio*) butterflies in the *Princeps nireus* group, which are endemic to eastern and central Africa, have dark wings with bright blue or blue-green dorsal wing bands or patches (fig. S1). The wing scales from their colored regions make up a nanostructure that is characterized by a ~2-μm-thick 2D photonic crystal slab (PCS) of hollow air cylinders in a medium of solid cuticle (Fig. 1, A and B). The cylinders have a mean diameter of ~240 nm and a spacing of ~340 nm. The PCS rests parallel to and ~1.5 μm above a three-layer, cuticle-based DBR, which forms the base of the scale (fig. S2). Highly fluorescent pigment is infused exclusively throughout the PCS; its peak emission wavelength is ~505 nm, depending on species, with peak excitation at ~420 nm (fig. S3). The arrangement of air cylinders within the PCS is quasiperiodic, made up of domains of triangular symmetry over a range exceeding several lattice constants (fig. S4A). Two-dimensional Fourier transforms of this structure confirm such quasiperiodicity, revealing a single principal component of spatial variation in refractive index (fig. S4B). This demonstrates the in-plane directional independence with which this PCS scatters light.

Because photonic band diagrams indicate all possible electromagnetic scattering interactions in periodic systems, we calculated the photonic band structure for the idealized intra-domain triangular symmetry in this crystal. We found that the peak fluorescence emission lies across a frequency band in which the density of accessible optical states is significantly depleted, i.e., the pseudo-gap region (Fig. 1C). This finding indicates that the 2D photonic crystal inhibits emission in the crystal plane and thereby increases its out-of-plane emission. To verify this, fluorescence emission was measured both with and without specimen immersion in index-matching fluid (immersion effectively removes the nanostructure's photonic influence). Time-resolved analysis yielded the fluorescence decay rate and the explicit influence of the PCS. Because decay rate depends on the local density of optical states (LDOS) (described by Fermi's Golden Rule), the immersion of a fluorescent photonic crystal in matching fluid will change the LDOS and, subsequently, the fluorescence lifetimes [previously demonstrated in synthetic photonic crystals (*1*)]. We found that immersion in matching fluids considerably modified the decay lifetime of fluorescent emission from the butterfly wing scales, principally around the peak emission wavelength. The primary decay lifetime increased from 0.43 to 0.58 ns in the case of *Papilio nireus* (fig. S5).

As in ultra–high-efficiency LEDs (*2*), these butterflies' DBRs support a spectral stop band that matches the peak emission from the structure above it. The DBRs reflect upwardly the downward-emitted fluorescence concurrently with nonabsorbed longer wavelengths pass through the PCS. The spatial separation between the DBR and PCS minimizes losses via coupling to guided modes in the DBR.

Excitation for this fluorescent material appears to be optimized for the radiance from blue skylight, which peaks around 420 nm. Additionally, because the α-absorbance band of rhodopsin (*3*) dominates the green wavelength photosensitivity of *Papilio* vision, the spectral form of this absorption is ideally placed for stimulation by fluorescence from conspecific wings (fig. S3). As with some shrimps (*4*) and birds (*5*), this enhances signaling, because absorption of visually less-productive short wavelengths leads to the emission of longer wavelengths that trigger photoreception.

References

1. W. L. Vos, A. Polman, *Mater. Res. Soc. Bull.* **26**, 642 (2001).
2. A. Erchak *et al.*, *Appl. Phys. Lett.* **78**, 563 (2001).
3. A. Kelber, C. Thunell, K. Arikawa, *J. Exp. Biol.* **204**, 2469 (2001).
4. C. H. Mazel, T. W. Cronin, R. L. Caldwell, N. J. Marshall, *Science* **303**, 51 (2004).
5. K. E. Arnold, I. P. F. Owens, N. J. Marshall, *Science* **295**, 92 (2002).

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5751/1151/DC1
Figs. S1 to S5
References

27 June 2005; accepted 30 September 2005
10.1126/science.1116612

School of Physics, Exeter University, Exeter EX4 4QL, UK.

*To whom correspondence should be addressed. E-mail: p.vukusic@ex.ac.uk

Logic of the Yeast Metabolic Cycle: Temporal Compartmentalization of Cellular Processes

Benjamin P. Tu, Andrzej Kudlicki, Maga Rowicka, Steven L. McKnight*

Budding yeast grown under continuous, nutrient-limited conditions exhibit robust, highly periodic cycles in the form of respiratory bursts. Microarray studies reveal that over half of the yeast genome is expressed periodically during these metabolic cycles. Genes encoding proteins having a common function exhibit similar temporal expression patterns, and genes specifying functions associated with energy and metabolism tend to be expressed with exceptionally robust periodicity. Essential cellular and metabolic events occur in synchrony with the metabolic cycle, demonstrating that key processes in a simple eukaryotic cell are compartmentalized in time.

Periodic behavior is prevalent in nature. One of the most intriguing examples of this phenomenon is circadian rhythm driven by biological clocks, found in nearly all kingdoms of life. Circadian rhythms allow organisms to coordinate their physiology with day-night cycles and may have first evolved to control cellular metabolism (*1*).

Similarly, the budding yeast *Saccharomyces cerevisiae* exhibits "cycles" in the form of glycolytic and respiratory oscillations (*2*). Such cycles were first documented over 40 years ago and can occur with a variety of period lengths both in cell-free extracts and during continuous culture (*3*–*12*). A recent study has described a ~40-min respiratory oscillation that produces a genome-wide, low-amplitude oscillation of transcription during continuous culture (*10, 12*). However, the molecular underpinnings responsible for controlling metabolic oscillation remain poorly understood.

We used a continuous culture system to reveal a robust, metabolic cycle in budding yeast. Here, we describe a yeast metabolic cycle (YMC) that drives the temporal, genome-wide transcription and coordination of essential cellular and metabolic processes in a manner reminiscent of the circadian cycle.

An ultradian metabolic cycle in yeast. We conducted our studies with the prototrophic, genetically tractable, diploid yeast strain CEN.PK (*13*). After growth to high density [optical density (OD_{600}) about 8 to 9] followed by a brief starvation period, the culture spontaneously began respiratory cycles as measured by oxygen consumption (Fig. 1). These highly robust cycles were about 4 to 5 hours in length and persisted indefinitely when the cultures were continuously supplemented with low concentrations of glucose. Each cycle was characterized by a reductive, nonrespiratory phase followed by an oxidative, respiratory phase wherein the synchronized culture rapidly consumed molecular oxygen (Fig. 1).

To understand the molecular basis of these metabolic cycles, we performed microarray analysis of gene expression and assessed whether any genes were expressed periodically. Total RNA was prepared every ~25 min over three consecutive cycles (*14*). The high sampling rate allowed determination of the periodicities of expressed genes, including genes that are expressed only very transiently (*14*). The temporal expression profiles of all yeast open reading frames (ORFs) are shown in Fig. 2. By using a periodicity algorithm (*14*), we determined that over half of yeast genes (~3552) exhibited periodic expression patterns at a confidence level of 95% (Fig. 2C). Not surprisingly, the most common period of transcript oscillation was ~300 min (Fig. 2C), the length of one respiratory cycle. Although transcript oscillations cycled with a period of ~300 min almost without exception, different genes were expressed maximally at entirely different times during the metabolic cycle (Fig. 2, A and B). Thus, the YMC is accompanied by a highly organized transcriptional cycle.

Genes encoding proteins associated with energy, metabolism, and protein synthesis were overrepresented in the list of periodic genes (Table 1) (*14*). Moreover, characterization of the periodic genes with the yeast proteome localization data (*15*) indicated that gene products localized to the mitochondria, cell periphery, and bud neck tended to be expressed periodically (Table 1). Of the 100 genes that exhibited the most periodic expression patterns, about two-thirds are nuclear-encoded genes involved in mitochondrial function (Table 2) (*14*). Taken together, these findings suggest that respiratory cycling is accompanied by cycles in metabolism and that variation in mitochondrial function is an important component of the YMC.

Cluster analysis. We turned to the most periodic genes as sentinels for the identification of clusters of genes having similar temporal expression patterns. For example, *MRPL10*, which encodes a mitochondrial ribosomal protein, is one of the most periodic genes, and its expression peaks when cells begin to cease oxygen consumption (Fig. 2B). With the use of *MRPL10* as a guide gene, we used clustering analysis to reveal a large number of genes that exhibit highly similar expression patterns to *MRPL10* (Fig. 3A and table S1) (*14*). Many genes within this cluster also encode components of mitochondrial ribosomes (Fig. 3A). On expanding our analysis to other annotated mitochondrial ribosomal genes, we found that 73 of 74 nuclear-encoded mitochondrial ribosomal genes displayed an extremely similar temporal expression pattern (fig. S1). The extent of coordinated expression of these genes was highest shortly after the cells ceased oxygen consumption (Fig. 3A), suggesting that

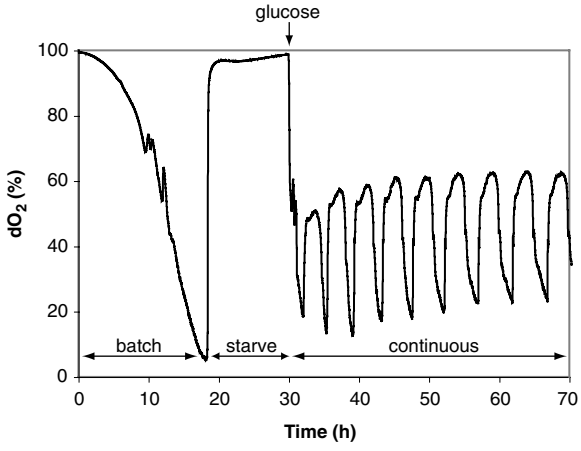
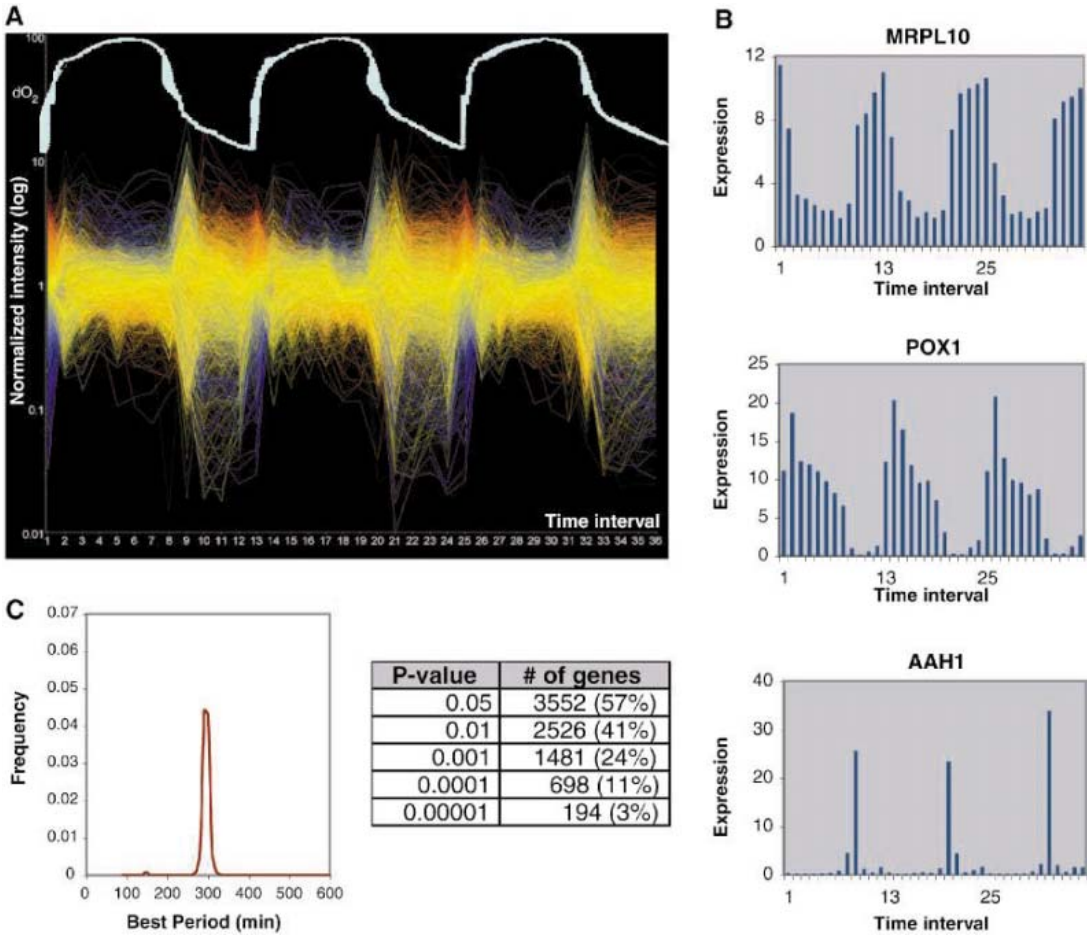


Fig. 1. The metabolic cycle of yeast. During batch mode, the cells are grown to a high density and then starved for at least 4 hours. During continuous mode (arrow), media containing glucose is introduced to the culture at a constant dilution rate (~0.09 to 0.1 $hours^{-1}$). dO_2 refers to dissolved oxygen concentrations (% saturation) in the media.

Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, L3.124, Dallas, TX 75390, USA.

*To whom correspondence should be addressed. E-mail: smckni@biochem.swmed.edu



P-value	# of genes
0.05	3552 (57%)
0.01	2526 (41%)
0.001	1481 (24%)
0.0001	698 (11%)
0.00001	194 (3%)

Fig. 2. Periodic gene expression during the metabolic cycle. (**A**) The expression profiles of ~6209 unique expressed ORFs are displayed (three cycles, 12 time intervals per cycle, ~25 min per time interval) beneath the dO_2 trace. Dissolved oxygen increased during time intervals 1 to 7, 13 to 19, and 25 to 31 and decreased during intervals 8 to 12, 20 to 24, and 32 to 36. Spectral coloring scheme of genes has its basis in relative expression at the start of reductive phase (red indicates higher; blue, lower). (**B**) The raw expression data for three individual genes: *MRPL10* (mitochondrial ribosomal protein of the large subunit), *POX1* (fatty-acyl CoA oxidase), and *AAH1* (adenine deaminase). Expression amount is in arbitrary units. (**C**) Periodicity analysis of gene expression. The most common period of transcript oscillation is ~300 min, the length of one cycle (*14*). The number of unique periodic genes at selected significance levels is shown (*14*).

cells were either rebuilding or duplicating their mitochondria at this time.

Extending the use of sentinel genes, we next turned to *POX1*, which encodes a peroxisomal fatty-acyl coenzyme A (CoA) oxidase. The peak of *POX1* gene expression occurs as dissolved oxygen accumulates during the YMC (Fig. 2B). By using *POX1* as a guide, we identified a cluster of genes with highly similar temporal expression patterns, most of which are annotated as encoding proteins involved in fatty acid oxidation and peroxisomal function (Fig. 3B and table S2). The coordinated expression of these genes strongly suggests that fatty acid oxidation preferentially occurs when the cells are not respiring.

One of the most periodic genes required for the building of cytoplasmic ribosomes is *RPL17B*, which encodes a protein component of the large (60S) ribosomal subunit. Use of *RPL17B* as a sentinel revealed a cluster of genes expressed most abundantly within a narrow window of the YMC (Fig. 3C). The vast majority of genes in this cluster also encode either ribosomal proteins or proteins involved in translation (Fig. 3C and table S3). Moreover, nearly all genes encoding cytoplasmic ribosomal proteins exhibit a similar expression pattern of peaking while the cells are respiring (fig. S2).

Table 1. Classification of periodic genes. The three most overrepresented and underrepresented functional categories (χ^2 test, P value comparing periodic with nonperiodic = 4×10^{-11}) and localizations (χ^2 test, P value = 2.2×10^{-16}) of periodic genes (*14*) are listed. Numbers in parentheses denote actual/expected number of genes. Functional categories according to MIPS (Munich Information Center for Protein Sequences) top-level classification.

Overrepresented		Underrepresented
	MIPS function	
Energy (104/75)		Transcription (364/420)
Metabolism (510/460)		Protein binding (312/341)
Protein synthesis (145/123)		Protein fate (386/408)
	Localization	
Mitochondria (217/153)		Nucleolus (44/68)
Cell periphery (54/42)		Early Golgi (10/18)
Bud neck (50/40)		Nuclear periphery (16/25)

We next performed an unbiased *k*-means cluster analysis of the entire microarray data set (*14*), which revealed three superclusters of gene expression (Fig. 3D). We termed these three superclusters Ox (oxidative), R/B (reductive/building), and R/C (reductive/charging), thereby defining three major phases of the YMC. Each of these superclusters comprises distinct subclasses of genes that are periodically expressed and peak within a certain window of the YMC (Fig. 3D).

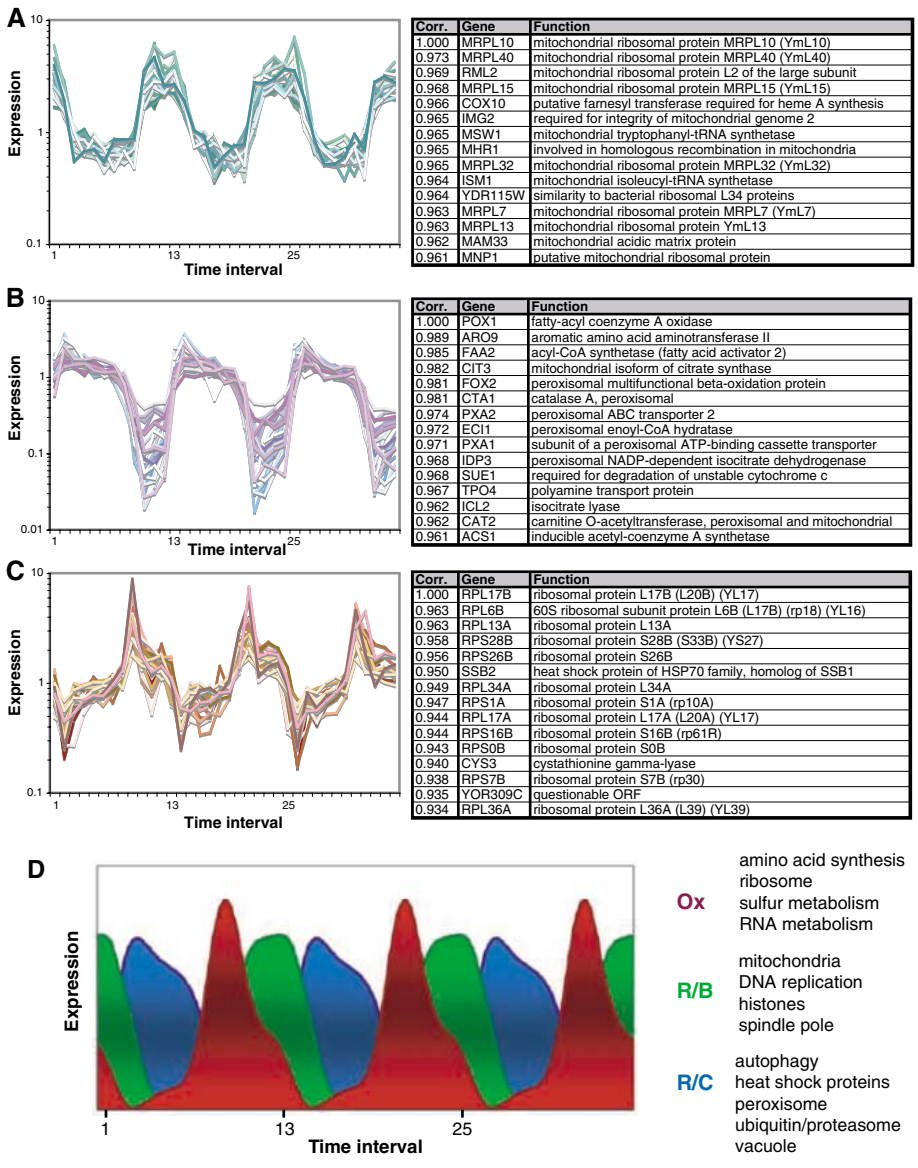
The Ox cluster consists primarily of genes encoding ribosomal proteins, translation initiation factors, amino acid biosynthetic enzymes, small nuclear RNAs, RNA processing enzymes, and proteins required for the uptake and metabolism of sulfur (Fig. 3D) (*14*). Because protein synthesis is one of the most energy-demanding processes (*16*), the translation machinery may be ideally assembled when abundant amounts of adenosine triphosphate (ATP) are readily available as a consequence of an intense burst of respiration. It is particularly surprising that the Ox supercluster contains many genes that peak extremely abruptly at a single time interval in the cycle (Figs. 2 and 3C). These transcripts must be very short-lived, helping to ensure an

exceptionally tight coupling of their anabolic role with presumed access to ATP resulting from oxidative phosphorylation.

Genes of the R/B supercluster peak when cells begin to cease oxygen consumption. This supercluster consists primarily of nuclear-encoded mitochondrial genes as well as genes encoding histones, spindle pole components, and proteins required for DNA replication and cell division (Fig. 3D) (*14*). The majority of genes encoding mitochondrial proteins, such as those involved in mitochondrial DNA replication, respiration, and protein import, all peak during this R/B phase (*14*).

Lastly, genes expressed maximally during the R/C supercluster encode proteins involved in nonrespiratory modes of metabolism and protein degradation (Fig. 3D). Genes encoding components of the peroxisome, vacuole, proteasome, and ubiquitination machinery are selectively activated throughout most of the R/C phase (*14*).

The tight coordination of gene expression during the YMC allows prediction of regulatory motifs within gene clusters. In the *POX1*-defined peroxisomal cluster, unbiased analysis of the noncoding sequences of the top 25 clustered genes identified two potential regulatory motifs in their upstream activating sequences (UASs). Application of the MEME algorithm (*17*) identified 5′-WGCCGCCGW-3′ (where W is A or T) and 5′-TTGGGGTAAW-3′ as putative regulatory motifs at a high level of statistical significance (Fig. 4). Upon examining the mitochondrial ribosomal cluster, we observed no previously described motif in the promoter regions aside from long, A-rich sequences (Fig. 4), in which guanines were sparsely distributed within long adenine stretches. Inspection of the 3′ untranslated regions (3′ UTRs) of the 74 nuclear-encoded mitochondrial ribosomal genes revealed the putative regulatory sequence 5′-CNTGTANATA-3′ (where N is any base) in 73 of 74 genes (Fig. 4). This motif may represent a binding site for the RNA-binding protein Puf3p (*18*). Out of the 74 genes queried, the only gene (*PPE1*) lacking the conserved 3′ UTR motif was the one that failed to be expressed with proper temporal periodicity (fig. S1), raising the possibility that it may be misannotated (*19*). Further identification of conserved motifs in gene clusters may uncover previously unknown regulatory elements required for transcriptional and post-transcriptional coordination of the YMC.



Corr.	Gene	Function
1.000	MRPL10	mitochondrial ribosomal protein MRPL10 (YmL10)
0.973	MRPL40	mitochondrial ribosomal protein MRPL40 (YmL40)
0.969	RML2	mitochondrial ribosomal protein L2 of the large subunit
0.968	MRPL15	mitochondrial ribosomal protein MRPL15 (YmL15)
0.966	COX10	putative farnesyl transferase required for heme A synthesis
0.965	IMG2	required for integrity of mitochondrial genome 2
0.965	MSW1	mitochondrial tryptophanyl-tRNA synthetase
0.965	MHR1	involved in homologous recombination in mitochondria
0.965	MRPL32	mitochondrial ribosomal protein MRPL32 (YmL32)
0.964	ISM1	mitochondrial isoleucyl-tRNA synthetase
0.964	YDR115W	similarity to bacterial ribosomal L34 proteins
0.963	MRPL7	mitochondrial ribosomal protein MRPL7 (YmL7)
0.963	MRPL13	mitochondrial ribosomal protein YmL13
0.962	MAM33	mitochondrial acidic matrix protein
0.961	MNP1	putative mitochondrial ribosomal protein

Corr.	Gene	Function
1.000	POX1	fatty-acyl coenzyme A oxidase
0.989	ARO9	aromatic amino acid aminotransferase II
0.985	FAA2	acyl-CoA synthetase (fatty acid activator 2)
0.982	CIT3	mitochondrial isoform of citrate synthase
0.981	FOX2	peroxisomal multifunctional beta-oxidation protein
0.981	CTA1	catalase A, peroxisomal
0.974	PXA2	peroxisomal ABC transporter 2
0.972	ECI1	peroxisomal enoyl-CoA hydratase
0.971	PXA1	subunit of a peroxisomal ATP-binding cassette transporter
0.968	IDP3	peroxisomal NADP-dependent isocitrate dehydrogenase
0.968	SUE1	required for degradation of unstable cytochrome c
0.967	TPO4	polyamine transport protein
0.962	ICL2	isocitrate lyase
0.962	CAT2	carnitine O-acetyltransferase, peroxisomal and mitochondrial
0.961	ACS1	inducible acetyl-coenzyme A synthetase

Corr.	Gene	Function
1.000	RPL17B	ribosomal protein L17B (L20B) (YL17)
0.963	RPL6B	60S ribosomal subunit protein L6B (L17B) (rp18) (YL16)
0.963	RPL13A	ribosomal protein L13A
0.958	RPS28B	ribosomal protein S28B (S33B) (YS27)
0.956	RPS26B	ribosomal protein S26B
0.950	SSB2	heat shock protein of HSP70 family, homolog of SSB1
0.949	RPL34A	ribosomal protein L34A
0.947	RPS1A	ribosomal protein S1A (rp10A)
0.944	RPL17A	ribosomal protein L17A (L20A) (YL17)
0.944	RPS16B	ribosomal protein S16B (rp61R)
0.943	RPS0B	ribosomal protein S0B
0.940	CYS3	cystathionine gamma-lyase
0.938	RPS7B	ribosomal protein S7B (rp30)
0.935	YOR309C	questionable ORF
0.934	RPL36A	ribosomal protein L36A (L39) (YL39)

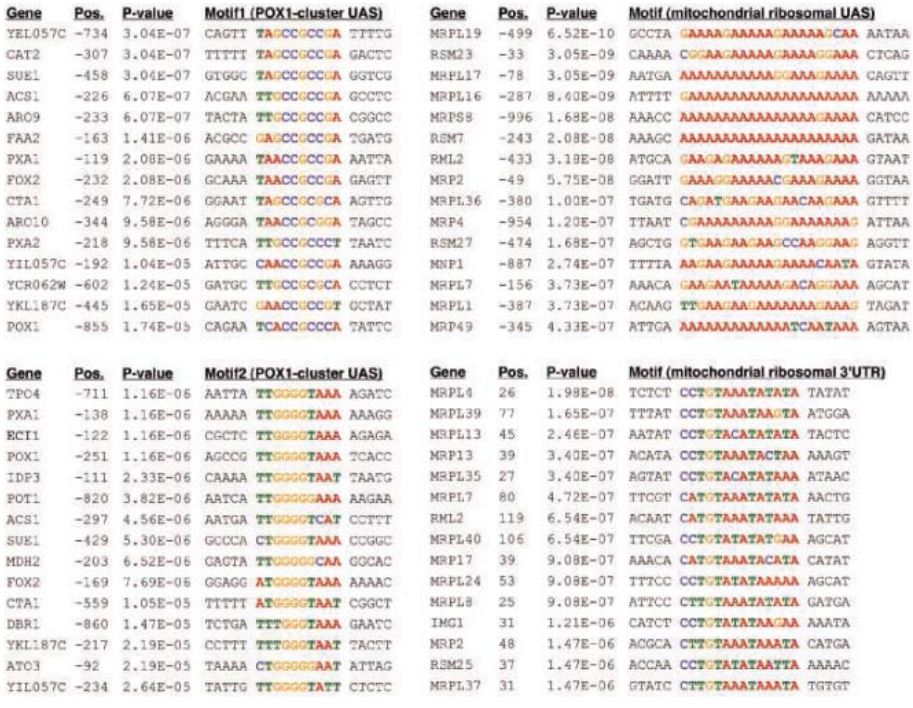
Fig. 3. Cluster analysis of gene expression during the metabolic cycle. (**A** to **C**) The mitochondrial ribosomal, peroxisomal, and ribosomal clusters. The expression profiles of the top 25 genes in each cluster are plotted together, and the identities and statistical correlations of the top 15 genes are listed to the right. Expression amount is in arbitrary units. (**D**) Average profiles for the three superclusters of gene expression during the metabolic cycle: Ox (oxidative), R/B (reductive, building), and R/C (reductive, charging) (*14*). The Ox supercluster (1023 genes) peaks roughly during time intervals 8 to 12, 20 to 24, and 32 to 36; the R/B supercluster (977 genes), during time intervals 10 to 14 and 22 to 26; and the R/C supercluster (1510 genes), during time intervals 2 to 7, 14 to 19, and 26 to 31. Examples of classes of genes in each supercluster are listed. For a complete list of genes in each supercluster, see (*14*).

The cell division cycle. We next chose as a sentinel the exceptionally periodic gene *YOX1* (Table 2), which encodes a homeodomain-containing transcriptional repressor involved in the cell cycle (*20*). Many genes involved in DNA replication and the cell cycle displayed highly similar temporal expression profiles, raising the possibility of a coupling between cell division and the YMC (Fig. 5A). Collectively, expression of these genes peaks in the R/B phase (Fig. 5A), suggesting that DNA replication and the cell division cycle may initiate during the very late Ox phase and proceed into the R/B phase of the YMC. Cell division is strictly confined to the reductive, nonrespiratory phases (Fig. 5B). Almost no cells were observed to either replicate DNA or divide during the Ox phase (Fig. 5B). Visual examination of cells at frequent intervals showed small buds beginning to appear at the very end of the Ox phase and synchronously growing larger during progression through the R/B and R/C phases (Fig. 5B). Almost no buds were observed in the midst of Ox phase (Fig. 5B). We estimated from both fluorescence-activated cell sorting (FACS) analysis and microscopic calculation of budded cells that roughly 50% of the diploid cells proceed through the cell cycle during each metabolic cycle (Fig. 5B). This value approximates the macroscopic behavior of the culture. On the basis of the dilution rate by fresh media, about half of all cells must divide each metabolic cycle to ensure stasis of culture mass. Confining the cell cycle to the reductive phases of the YMC may allow cells to minimize oxidative damage to DNA.

Dynamic changes during the metabolic cycle. The periodic transcription of genes during the YMC can be predicted to drive periodic fluctuations in metabolic output. By using ^{1}H

Table 2. The 40 most periodic genes, ranked by periodicity score (*14*).

Gene	*P* value	Function
MSS116	2.77×10^{-7}	Mitochondrial RNA helicase of the DEAD box family
MEF1	4.57×10^{-7}	Mitochondrial elongation factor G-like protein
BCS1	5.15×10^{-7}	Mitochondrial protein of the CDC48/PAS1/SEC18 ATPase family
MRPL10	5.42×10^{-7}	Mitochondrial ribosomal protein MRPL10 (YmL10)
ISM1	5.56×10^{-7}	Mitochondrial isoleucyl-tRNA synthetase
YLR253W	6.29×10^{-7}	Weak similarity to bacterial aminoglycoside acetyltransferase regulators
MRPL40	6.79×10^{-7}	Mitochondrial ribosomal protein MRPL40 (YmL40)
FIT2	7.40×10^{-7}	Cell wall mannoprotein
PRY2	7.45×10^{-7}	Similar to plant PR-1 class of pathogen-related proteins
COX10	8.06×10^{-7}	Farnesyl transferase required for heme A synthesis
MRPL32	8.14×10^{-7}	Mitochondrial ribosomal protein MRPL32 (YmL32)
MRPL4	8.81×10^{-7}	Mitochondrial 60S ribosomal protein L4 (YmL4)
YER163C	8.81×10^{-7}	Weak similarity to *Escherichia coli* cation transport protein
YML6	9.59×10^{-7}	Mitochondrial ribosomal protein
MSD1	1.04×10^{-6}	Mitochondrial aspartyl-tRNA synthetase
YJL213W	1.05×10^{-6}	Similarity to *Methanobacterium* aryldialkylphosphatase related protein
MRPL23	1.07×10^{-6}	Mitochondrial ribosomal protein of the large subunit (YmL23)
SRL1	1.09×10^{-6}	Suppressor of rad53 lethality, cell wall mannoprotein
YDR493W	1.09×10^{-6}	Hypothetical protein
YNL300W	1.10×10^{-6}	Hypothetical protein
MRPL19	1.13×10^{-6}	Mitochondrial ribosomal protein of the large subunit (YmL19)
MTO1	1.22×10^{-6}	Mitochondrial protein
MRPL11	1.23×10^{-6}	Mitochondrial ribosomal protein MRPL11 (YmL11)
YPL103C	1.24×10^{-6}	Similarity to hypothetical *Mycobacterium tuberculosis* protein
MSW1	1.28×10^{-6}	Mitochondrial tryptophanyl-tRNA synthetase
CBP3	1.30×10^{-6}	Required for assembly of ubiquinol cytochrome-c reductase complex
SCW10	1.30×10^{-6}	Member of the glucanase gene family
YOX1	1.33×10^{-6}	Homeobox domain–containing transcriptional repressor
MRPL7	1.33×10^{-6}	Mitochondrial ribosomal protein MRPL7 (YmL7)
PET117	1.37×10^{-6}	Cytochrome c oxidase assembly factor
MRPL35	1.43×10^{-6}	Mitochondrial ribosomal protein MRPL35 (YmL35)
YJL051W	1.45×10^{-6}	Hypothetical protein
MRPL13	1.46×10^{-6}	Mitochondrial ribosomal protein YmL13
YFL052W	1.48×10^{-6}	Strong similarity to Mal63p, YPR196w, and Mal13p
CRC1	1.52×10^{-6}	Mitochondrial inner membrane carnitine transporter
RML2	1.58×10^{-6}	Mitochondrial ribosomal protein L2 of the large subunit
MSE1	1.59×10^{-6}	Mitochondrial glutamyl-tRNA synthetase
RSM19	1.62×10^{-6}	Mitochondrial ribosomal protein of the small subunit
TOS4	1.73×10^{-6}	Transcription factor, induced in G1 by SBF
MRPL31	1.75×10^{-6}	Mitochondrial ribosomal protein YmL31



Gene	Pos.	P-value	Motif1 (POX1-cluster UAS)
YEL057C	-734	3.04E-07	CAGTT TAGCCGCCGA TTTTG
CAT2	-307	3.04E-07	TTTTT TAGCCGCCGA GACTC
SUE1	-458	3.04E-07	GTGGC TAGCCGCCGA GGTCG
ACS1	-226	6.07E-07	ACGAA TTGCCGCCGA GCCTC
ARO9	-233	6.07E-07	TACTA TTGCCGCCGA CGGCC
FAA2	-163	1.41E-06	ACGCC GAGCCGCCGA TGATG
PXA1	-119	2.08E-06	GAAAA TAACCGCCGA AATTA
FOX2	-232	2.08E-06	GCAAA TAACCGCCGA GAGTT
CTA1	-249	7.72E-06	GGAAT TAGCCGCGCA AGTTG
ARO10	-344	9.58E-06	AGGGA TAACCGCGGA TAGCC
PXA2	-218	9.58E-06	TTTCA TTGCCGCCCT TAATC
YIL057C	-192	1.04E-05	ATTGC CAACCGCCGA AAAGG
YCR062W	-602	1.24E-05	GATGC TTGCCGCGCA CCTCT
YKL187C	-445	1.65E-05	GAATC GAACCGCCGT GCTAT
POX1	-855	1.74E-05	CAGAA TCACCGCCCA TATTC

Gene	Pos.	P-value	Motif (mitochondrial ribosomal UAS)
MRPL19	-499	6.52E-10	GCCTA GAAAAGAAAAAGAAAAAGCAA AATAA
RSM23	-33	3.05E-09	CAAAA CGGAAGAAAAAGAAAAGGAAA CTCAG
MRPL17	-78	3.05E-09	AATGA AAAAAAAAAAAGGAAAGAAAA CAGTT
MRPL16	-287	8.40E-09	ATTTT GAAAAAAAAAAAAAAAAAAAA AAAAA
MRPS8	-996	1.68E-08	AAACC AAAAAAAAAAAAAAAAGAAAA CATCC
RSM7	-243	2.08E-08	AAAGC AAAAAAAAAAAAAAAAAAAAA GATAA
RML2	-433	3.18E-08	ATGCA GAACAGAAAAAAGTAAAGAAA GTAAT
MRP2	-49	5.75E-08	GGATT GAAAGGAAAAACGAAAGAAAA GGTAA
MRPL36	-380	1.00E-07	TGATG CAGATGAAGAAGAACAAGAAA GTTTT
MRP4	-954	1.20E-07	TTAAT CGAAAAAAAAAGGAAAAAAAG ATTAA
RSM27	-474	1.68E-07	AGCTG GTGAAGAAGAAGCCAAGGAAG AGGTT
MNP1	-887	2.74E-07	TTTTA AAGAAGAAAAAGAAAACAATA GTATA
MRPL7	-156	3.73E-07	AAACA GAAGAATAAAAAGACAGGAAA AGCAT
MRPL1	-387	3.73E-07	ACAAG TTGAAGAAGAAAAAAAGAAAG TAGAT
MRP49	-345	4.33E-07	ATTGA AAAAAAAAAAAAATCAATAAA AGTAA

Gene	Pos.	P-value	Motif2 (POX1-cluster UAS)
TPO4	-711	1.16E-06	AATTA TTGGGGTAAA AGATC
PXA1	-138	1.16E-06	AAAAA TTGGGGTAAA AAAGG
ECI1	-122	1.16E-06	CGCTC TTGGGGTAAA AGAGA
POX1	-251	1.16E-06	AGCCG TTGGGGTAAA TCACC
IDP3	-111	2.33E-06	CAAAA TTGGGGTAAT TAATG
POT1	-820	3.82E-06	AATCA TTGGGGGAAA AAGAA
ACS1	-297	4.56E-06	AATGA TTGGGGTCAT CCTTT
SUE1	-429	5.30E-06	GCCCA CTGGGGTAAA CCGGC
MDH2	-203	6.52E-06	CAGTA TTGGGGGCAA GGCAC
FOX2	-169	7.69E-06	GGAGG ATGGGGTAAA AAAAC
CTA1	-559	1.05E-05	TTTTT ATGGGGTAAT CGGCT
DBR1	-860	1.47E-05	TCTGA TTTGGGTAAA GAATC
YKL187C	-217	2.19E-05	CCTTT TTTGGGTAAT TACTT
ATO3	-92	2.19E-05	TAAAA CTGGGGGAAT ATTAG
YIL057C	-234	2.64E-05	TATTG TTGGGGTATT CTCTC

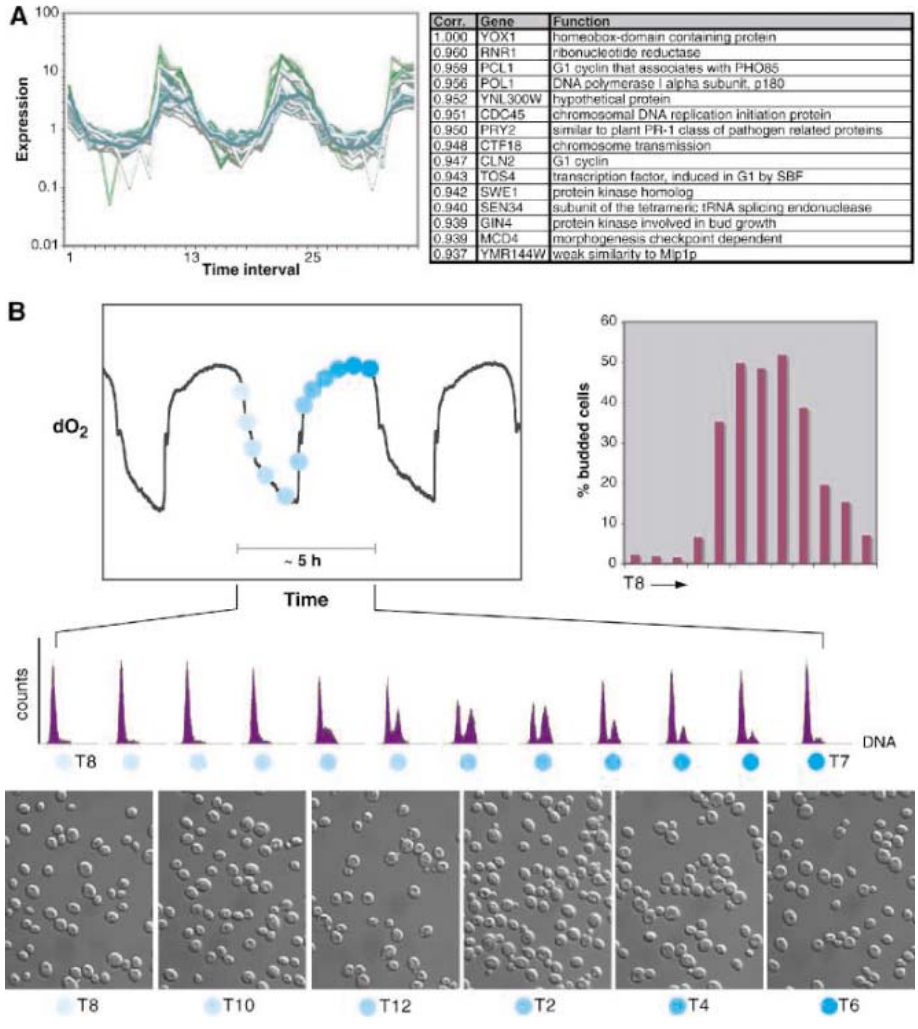
Gene	Pos.	P-value	Motif (mitochondrial ribosomal 3'UTR)
MRPL4	26	1.98E-08	TCTCT CCTGTAAATATATA TATAT
MRPL39	77	1.65E-07	TTTAT CCTGTAAATAAGTA ATGGA
MRPL13	45	2.46E-07	AATAT CCTGTACATATATA TACTC
MRP13	39	3.40E-07	ACATA CCTGTAAATACTAA AAAGT
MRPL35	27	3.40E-07	AGTAT CCTGTACATATAAA ATAAC
MRPL7	80	4.72E-07	TTCGT CATGTAAATATATA AACTG
RML2	119	6.54E-07	ACAAT CATGTAAATATAAA TATTG
MRPL40	106	6.54E-07	TTCGA CCTGTATATATGAA AGCAT
MRP17	39	9.08E-07	AAACA CATGTAAATACATA CATAT
MRPL24	53	9.08E-07	TTTCC CCTGTATATAAAAA AGCAT
MRPL8	25	9.08E-07	ATTCC CTTGTAAATATATA GATGA
IMG1	31	1.21E-06	CATCT CCTGTATATAAGAA AAATA
MRP2	48	1.47E-06	ACGCA CTTGTAAATAAATA CATGA
RSM25	37	1.47E-06	ACCAA CCTGTATATAATTA AAAAC
MRPL37	31	1.47E-06	GTATC CTTGTAAATAAATA TGTGT

Fig. 4. Prediction of regulatory motifs. Two putative motifs were found in the UAS of genes in the peroxisomal cluster (motif1 in 16 of 25 genes and motif2 in 17 of 25 genes), and one putative motif was found in both the UAS (74 of 74 genes) and the 3′ UTR of nuclear-encoded mitochondrial ribosomal genes (73 of 74 genes). Pos., position.

nuclear magnetic resonance (NMR), we determined that ethanol and acetate concentrations in the media fluctuate in a robust and periodic manner during the YMC (Fig. 6A), peaking near the end of Ox phase and the beginning of R/B phase (Fig. 6A). At this time, cells have mostly finished respiration, and the onset of cell division and cellular building may favor glycolytic, fermentative metabolism. Accordingly, the expression of the gene encoding alcohol dehydrogenase *ADH1* begins to increase shortly before the rise in ethanol concentrations (*14*).

Many genes involved in glycolysis and the breakdown of storage carbohydrates peak in expression during the R/C phase (Fig. 6B). Furthermore, genes involved in mobilizing ethanol for the citric acid (TCA) cycle are also selectively expressed during the R/C phase of the YMC. These include *ADH2*, the alcohol dehydrogenase that converts ethanol to acetaldehyde, and *ASC1* the acetyl-CoA synthetase enzyme (Fig. 6B). The net result of these metabolic reactions is the temporally limited accumulation of acetyl-CoA (Fig. 6B). Moreover, the concurrent oxidation of fatty acids during the R/C phase should result in the production of additional acetyl-CoA units during the R/C window of the YMC (Fig. 3A). In sum, cellular metabolism during the reductive phase is predicted to be heavily devoted to the production of acetyl-CoA, preparing cells for the upcoming Ox phase. During the Ox phase, metabolism shifts to respiration as accumulated acetyl-CoA units are used for ATP production via the TCA cycle and the electron transport chain.

Metabolic output in the R/C phase would also be predicted to optimize production of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) through the induction of *GND2* and the pentose phosphate pathway (Fig. 6B). Not only is *GND2* upregulated, but the genes encoding two enzymes (transketolase and transaldolase) that convert five-carbon sugars back to glycolytic intermediates are also coordinately activated (Fig. 6B). Both moles of NADPH made per mole of sugar are created before the return of pentose sugars to glycolysis (*21*). Apparently, transcriptional regulation of the genes encoding these enzymes helps tune metabolism for production of NADPH during the R/C phase. The NADPH produced is anticipated to buffer cells against oxidative stress associated with respiration. The highly concerted organization of gene expression driving the production of acetyl-CoA and NADPH allows characterization of this as the charging (R/C) phase of the yeast metabolic cycle. Lastly, ^{1}H NMR studies revealed almost no glucose in the media during any time of the metabolic cycle (*22*). Despite continuous infusion of the sugar, cells appear to immediately adsorb and metabolize available glucose.



Corr.	Gene	Function
1.000	YOX1	homeobox-domain containing protein
0.960	RNR1	ribonucleotide reductase
0.959	PCL1	G1 cyclin that associates with PHO85
0.956	POL1	DNA polymerase I alpha subunit, p180
0.952	YNL300W	hypothetical protein
0.951	CDC45	chromosomal DNA replication initiation protein
0.950	PRY2	similar to plant PR-1 class of pathogen related proteins
0.948	CTF18	chromosome transmission
0.947	CLN2	G1 cyclin
0.943	TOS4	transcription factor, induced in G1 by SBF
0.942	SWE1	protein kinase homolog
0.940	SEN34	subunit of the tetrameric tRNA splicing endonuclease
0.939	GIN4	protein kinase involved in bud growth
0.939	MCD4	morphogenesis checkpoint dependent
0.937	YMR144W	weak similarity to Mlp1p

Fig. 5. Gating of the cell cycle by the metabolic cycle. (**A**) DNA replication and cell cycle genes cluster together. The expression profiles of the top 25 clustered genes are plotted together, and the identities and statistical correlations of the top 15 genes are listed to the right. (**B**) DNA replication and cell division occur only during the reductive phases of the YMC. FACS analysis of the cell population was performed at 12 time intervals over the metabolic cycle. Differential interference contrast microscopy images of the population were used to count the percentage of budded cells (minimum 300 counted per time interval, images at every other interval are shown). Counts measured by number of cells; DNA was measured by propidium iodide signal.

The large amplitude of metabolic fluctuation between oxidative and reductive phases of the YMC predicts that intracellular redox states should vary considerably as a function of the cycle. The redox-sensitive transcription factor Yap1p translocates to the nucleus upon exposure of cells to oxidative stress (*23*). With use of a *YAP1*– green fluorescent protein (GFP) fusion, we monitored the intracellular localization of Yap1p during the YMC. This reporter indicated nuclear localization of Yap1p during the peak of oxidative respiration (Fig. 6C). By contrast, Yap1p appeared to be exclusively cytosolic at other times of the metabolic cycle (Fig. 6C). These observations suggest that, during the Ox phase, the intracellular redox state becomes notably more oxidized, although the differences in Yap1p localization could also be due to varying concentrations of the glycolytic metabolite methylglyoxal (*24*). Altered redox states within a cell may thus be a hallmark of different stages of the YMC.

We next analyzed global organelle morphology across the YMC by transmission electron microscopy (TEM) (*14*). The vacuole was much more prominent in Ox phase than in R/B and R/C phases of the YMC (Fig. 6D). These studies also provided evidence of autophagy in the Ox phase as seen by cellular material located within the vacuole (Fig. 6D). We then constructed a strain containing a GFP–tagged version of *VPH1*, a vacuolar ATPase subunit that resides in the vacuolar membrane (*25*). Visualization of these cells confirmed that vacuolar morphology changes substantially during the YMC and showed that the vacuolar membrane was more defined during the late R/C and early Ox phases (Fig. 6D). At other times, it was more dispersed and often completely undefined (Fig. 6D). This timing of vacuole emergence is con-

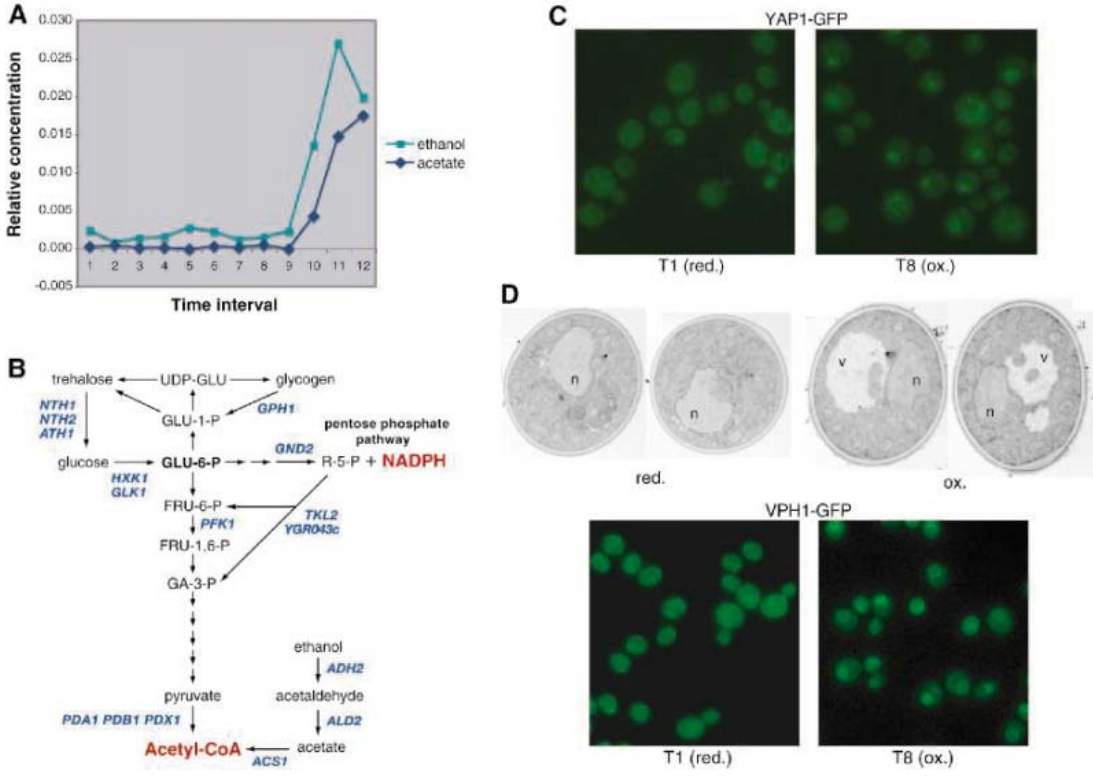


Fig. 6. Dynamic changes in metabolites, protein localization, and organelle morphology during the metabolic cycle. (**A**) Ethanol and acetate concentrations fluctuate during the metabolic cycle. Metabolites in the extracellular media were assayed by ^{1}H NMR (*14*). (**B**) Diagram outlining the predicted production of acetyl-CoA and NADPH during the reductive phase. Genes in blue are up-regulated notably during the R/C phase. (**C**) The metabolic cycle is a redox cycle. Cells expressing the redox-sensitive *YAP1*-GFP reporter were harvested at different times of the metabolic cycle. After fixation, Yap1p-GFP localization was visualized by fluorescence microscopy (T1, reductive; T8, oxidative) (*14*). (**D**) Dynamics of the vacuole during the metabolic cycle. (Top) TEM images of cells harvested during the reductive (red., T2) or oxidative (ox., T9) phase (n, nucleus; v, vacuole) (*14*). (Bottom) Images of cells expressing the vacuolar marker *VPH1*-GFP at different times of the cycle.

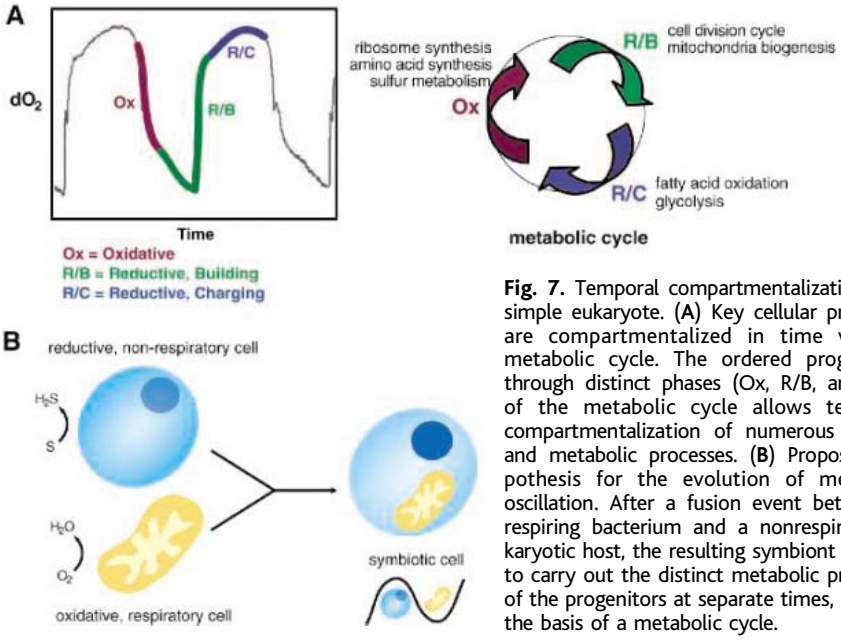


Fig. 7. Temporal compartmentalization in a simple eukaryote. (**A**) Key cellular processes are compartmentalized in time via the metabolic cycle. The ordered progression through distinct phases (Ox, R/B, and R/C) of the metabolic cycle allows temporal compartmentalization of numerous cellular and metabolic processes. (**B**) Proposed hypothesis for the evolution of metabolic oscillation. After a fusion event between a respiring bacterium and a nonrespiring eukaryotic host, the resulting symbiont evolved to carry out the distinct metabolic programs of the progenitors at separate times, forming the basis of a metabolic cycle.

sistent with results showing that genes involved in vacuolar trafficking, protein degradation, and autophagy peak during the R/C phase before respiration (Fig. 3D) (*14*). Thus, vacuole morphology and function are highly dynamic during the YMC. Vacuole-mediated catabolism and autophagy may be natural steps in the rebuilding of organelles and cytosol in preparation for the imminent R/B building phase.

Discussion. We have described here an unusually robust, ultradian cycle in budding yeast grown under nutrient-limited continuous conditions. Over half of the yeast genome is transcriptionally regulated in a rigidly periodic fashion as a function of this 4- to 5-hour cycle. This periodic gene expression is more robust than those seen during the circadian cycle of certain bacteria (*Synechococcus elongatus*), fungi (*Neurospora crassa*), and almost all metazoan organisms (*26–29*).

The YMC controls the timing of diverse and distinct cellular and metabolic processes: Respiration, mitochondria biogenesis, ribosome biogenesis, DNA replication, cell division, fatty acid oxidation, glycolysis, and vacuole-mediated catabolism are all predicted to be precisely compartmentalized in time (Fig. 7A). Such temporal orchestration may allow cells to perform anabolic and catabolic processes in a finely coordinated and efficient fashion, helping to minimize the occurrence of futile reactions. Accordingly, genes that have functions associated with energy and metabolism tend to be expressed periodically (Table 1). Thus, cells seem to generate ATP by distinct metabolic pathways, including mitochondrial respiration, glycolysis, and fatty acid oxidation, which operate in different temporal windows of the YMC (Fig. 7A). We have focused on only a small number of the dynamic cellular changes that are temporally orchestrated throughout the YMC. Further exploration of our data set will undoubtedly reveal additional examples of events that are executed during temporally restricted windows of the YMC.

Almost all cells in our culture system rapidly become synchronized with respect to both the YMC and the cell cycle during continuous culture. This conclusion can be drawn from both macroscopic data, such as oxygen consumption, and microarray data, which show that nearly all the ~3500 cycling transcripts exhibit a periodic-

ity precisely matching the length of the YMC. It is possible that a limited number of highly specialized, secreted metabolites, analogous to those regulating quorum sensing in bacteria (*30*) and present at cyclically fluctuating amounts in the culture medium, control YMC synchronization. Alternatively, synchrony may be generated by fluctuating amounts of a multitude of generic metabolites, including ethanol and acetate (Fig. 6A), a cross-coupling mechanism analogous to the multinucleated, syncytial state of slime molds such as *Physarum*.

Our continuous culture system, which spontaneously generates metabolic cycles, defines a physiological paradigm that probably reflects yeast growth in the wild. The dense population of cells in the fermentor may approximate a colony exposed to nutrient-limited growth. Under these conditions, yeast cells deploy regulatory capabilities that are not typical of laboratory strains grown at log phase in rich medium. Under common laboratory growth conditions, yeast do not usually perform mitochondrial respiration. Likewise, many classes of genes, including those encoding products required for fatty acid oxidation, have minimal log-phase expression (*31*). By contrast, almost all of the genes required for mitochondrial and peroxisomal function are expressed and coordinately activated during a precise temporal window of the YMC.

How are the oscillating gene expression patterns of the YMC coordinated? Either messenger RNA (mRNA) synthesis, turnover, or both must combine to yield the pervasively periodic fluctuations in transcript abundance. The conserved sequences in the promoter regions of genes expressed with matching temporal kinetics that we observed indicate that regulatory periodicity will be executed, at least in part, by gene-specific transcription factors. Indeed, the mRNAs for over 60% of annotated yeast transcription factors fluctuated in abundance as a function of the YMC (*14*). Transcription factors that do not change in abundance might still be involved in its selective regulation. Such transcription factors may possess intrinsic metabolic sensors or be themselves coupled to signaling systems endowed with the capability to sense redox or metabolic state. The YMC will be regulated at numerous posttranscriptional levels, including selective translation and turnover of mRNA, regulated protein degradation, phosphorylation-dependent signaling, and fundamental enzyme allostery.

Our data also indicate that both DNA replication and cell division are temporally regulated as a function of the YMC. That transcriptional regulation of genes gating replication and cell division is biologically meaningful is strongly supported by FACS analysis and quantitation of budding (Fig. 5, A and B). These data demonstrate that replication and cell division are restricted exclusively to the reductive phases of the YMC. Cells may have evolved this tight coupling to ensure that the cell cycle evades the potentially mutagenic redox environment of the oxidative respiratory phase. As shown in Fig. 7A, there is a conceptual relation between the tripartite Ox, R/B, and R/C oscillation of the metabolic cycle and the phases of the cell division cycle (G1, S, G2, and M). Such gating of cell division has been observed as a function of circadian or ultradian rhythm in other species (*11*, *32*–*34*).

What are the evolutionary origins of the YMC? About two-thirds of the most periodic transcripts in the YMC encode components of mitochondria (Table 2) (*12*). Thus, the timing of construction of new mitochondria, or reconstruction of spent mitochondria, is probably of exceptional importance to the YMC. These observations, coupled with the fact that mitochondrial respiration is restricted to a brief window during the YMC, highlight the ability of yeast cells to temporally regulate oxidative phosphorylation. Might it be that an ancestral symbiont (*33*), endowed with the capacity to generate ATP by both respiratory and reductive pathways, used the two pathways in an oscillatory manner (Fig. 7B)? Given the expectation that the two pathways were optimally tuned in the progenitors of the symbiont, we speculate that the resulting hybrid found a way of leaving each pathway close to its own optimal, physiological milieu by temporal compartmentalization.

Temporal compartmentalization of metabolic function also appears to take place during the circadian cycle of flies and mice (*25*, *26*). The primitive cyanobacterium *Synechococcus elongatus*, which conducts both photosynthesis and nitrogen fixation, uses its circadian regulatory apparatus to ensure that these biochemically incompatible pathways are executed at temporally distinct phases of the circadian cycle (*34*). The circadian cycle drives the periodic expression of many genes encoding the rate-limiting enzymes of numerous metabolic processes (*25*, *26*). Restricted feeding can entrain the circadian cycle (*35*, *36*), perhaps through metabolic feedback impinging directly on the transcription factors that themselves regulate circadian rhythm (*37*, *38*). Metabolic oscillation may, therefore, constitute the primordial device upon which the divergent circadian and ultradian biological oscillators of modern organisms have been built (*1*).

References and Notes

1. J. Rutter, M. Reick, S. L. McKnight, *Annu. Rev. Biochem.* **71**, 307 (2002).
2. P. Richard, *FEMS Microbiol. Rev.* **27**, 547 (2003).
3. B. Chance, R. W. Estabrook, A. Ghosh, *Proc. Natl. Acad. Sci. U.S.A.* **51**, 1244 (1964).
4. B. Chance, B. Schoener, S. Elsaesser, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 337 (1964).
5. A. Ghosh, B. Chance, *Biochem. Biophys. Res. Commun.* **16**, 174 (1964).
6. F. A. Hommes, *Arch. Biochem. Biophys.* **108**, 36 (1964).
7. H. K. von Meyenburg, *Arch. Microbiol.* **66**, 289 (1969).
8. D. Porro, E. Martegani, B. Ranzi, L. Alberghina, *Biotechnol. Bioeng.* **32**, 411 (1988).
9. A. D. Satroutdinov, H. Kuriyama, H. Kobayashi, *FEMS Microbiol. Lett.* **77**, 261 (1992).
10. Z. Palkova *et al.*, *Mol. Biol. Cell* **13**, 3901 (2002).
11. R. R. Klevecz, J. Bolen, G. Forrest, D. B. Murray, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1200 (2004).
12. D. Lloyd, D. B. Murray, *Trends Biochem. Sci.* **30(7)**, 373 (2005).
13. J. P. van Dijken *et al.*, *Enzyme Microb. Technol.* **26**, 706 (2000).
14. Materials and methods are available as supporting material on *Science* Online.
15. W. K. Huh *et al.*, *Nature* **425**, 686 (2003).
16. M. F. Princiotta *et al.*, *Immunity* **18**, 343 (2003).
17. T. L. Bailey, C. Elkan, in *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, Stanford, CA, 14 to 17 August 1994 (American Association for Artificial Intelligence, Menlo Park, CA, 1994), pp. 28–36.
18. A. P. Gerber, D. Herschlag, P. O. Brown, *PLoS Biol.* **2**, E79 (2004).
19. J. Wu *et al.*, *EMBO J.* **19**, 5672 (2000).
20. T. Pramila, S. Miles, D. GuhaThakurta, D. Jemiolo, L. L. Breeden, *Genes Dev.* **16**, 3034 (2002).
21. L. Stryer, *Biochemistry* (Freeman, New York, ed. 4, 1995), p. xxxiv.
22. B. P. Tu, A. Kudlicki, M. Rowicka, S. L. McKnight, data not shown.
23. S. Kuge, N. Jones, A. Nomoto, *EMBO J.* **16**, 1710 (1997).
24. K. Maeta, S. Izawa, S. Okazaki, S. Kuge, Y. Inoue, *Mol. Cell. Biol.* **24**, 8753 (2004).
25. E. Conibear, T. H. Stevens, *Methods Enzymol.* **351**, 408 (2002).
26. Y. Liu *et al.*, *Genes Dev.* **9**, 1469 (1995).
27. A. Claridge-Chang *et al.*, *Neuron* **32**, 657 (2001).
28. S. Panda *et al.*, *Cell* **109**, 307 (2002).
29. A. Correa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13597 (2003).
30. J. M. Henke, B. L. Bassler, *Trends Cell Biol.* **14**, 648 (2004).
31. S. Ghaemmaghami *et al.*, *Nature* **425**, 737 (2003).
32. T. Mori, B. Binder, C. H. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10183 (1996).
33. T. Matsuo *et al.*, *Science* **302**, 255 (2003); published online 21 August 2003 (10.1126/science.1086271).
34. E. Nagoshi *et al.*, *Cell* **119**, 693 (2004).
35. L. Margulis, *Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth* (Freeman, San Francisco, CA, 1981), p. xxii.
36. S. S. Golden, S. R. Canales, *Nat. Rev. Microbiol.* **1**, 191 (2003).
37. F. Damiola *et al.*, *Genes Dev.* **14**, 2950 (2000).
38. K. A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, M. Menaker, *Science* **291**, 490 (2001).
39. J. Rutter, M. Reick, L. C. Wu, S. L. McKnight, *Science* **293**, 510 (2001); published online 5 July 2001 (10.1126/science.1060698).
40. E. M. Dioum *et al.*, *Science* **298**, 2385 (2002); published online 20 November 2002 (10.1126/science.1078456).

41. We thank S. Fodor of Affymetrix, Incorporated for providing yeast microarrays; the University of Texas Southwestern Microarray Core Facility for assistance with microarray experiments; C. Malloy and the Rogers NMR Center for assistance with NMR; T. Januszewski for assistance with electron microscopy, H. Lai and L. Tu for assistance with illustrations; D. Wilson, L. Wu, and J. Longgood for technical assistance; Z. Otwinowski, R. Klevecz, D. Murray, J. Goodman, and J. Chen for helpful discussions; and P. Kötter for providing the CEN.PK strain. This work was supported by an NIH Director's Pioneer Award, unrestricted endowment funds from an anonymous donor (S.L.M.), and a Helen Hay Whitney Foundation postdoctoral fellowship (B.P.T.). Microarray data have been deposited with Gene Expression Omnibus (series accession GSE3431 and at http://yeast.swmed.edu

Supporting Online Material

www.sciencemag.org/cgi/content/full/1120499/DC1
Materials and Methods
Figs S1 and S2
Tables S1 to S3
References

30 June 2005; accepted 19 October 2005
Published online 27 October 2005;
10.1126/science.1120499
Include this information when citing this paper.

Structure of the Quaternary Complex of Interleukin-2 with Its α, β, and γ_c Receptors

Xinquan Wang,* Mathias Rickert,* K. Christopher Garcia†

Interleukin-2 (IL-2) is an immunoregulatory cytokine that acts through a quaternary receptor signaling complex containing alpha (IL-2Rα), beta (IL-2Rβ), and common gamma chain (γ_c) receptors. In the structure of the quaternary ectodomain complex as visualized at a resolution of 2.3 angstroms, the binding of IL-2Rα to IL-2 stabilizes a secondary binding site for presentation to IL-2Rβ. γ_c is then recruited to the composite surface formed by the IL-2/IL-2Rβ complex. Consistent with its role as a shared receptor for IL-4, IL-7, IL-9, IL-15, and IL-21, γ_c forms degenerate contacts with IL-2. The structure of γ_c provides a rationale for loss-of-function mutations found in patients with X-linked severe combined immunodeficiency diseases (X-SCID). This complex structure provides a framework for other γ_c-dependent cytokine-receptor interactions and for the engineering of improved IL-2 therapeutics.

The cytokine IL-2 is mainly produced by antigen-activated T cells and promotes the proliferation, differentiation, and survival of mature T and B cells as well as the cytolytic activity of natural killer (NK) cells in the innate immune defense (*1*, *2*). IL-2 is used therapeutically as an immune adjuvant in certain types of lymphoproliferative diseases and cancers, and IL-2 antagonists can prevent organ transplant rejection (*3*, *4*). However, severe dose-limiting toxicity has limited its effectiveness in the clinic. These deleterious side effects are mediated through different combinations of IL-2 receptors, which suggests that structure-based engineering of receptor-selective variants could have clinical benefit (*5*).

IL-2 exerts its pleiotropic activities through binding to different receptor complexes, depending on which of the components are expressed on the cell surface: the alpha chain (IL-2Rα), beta chain (IL-2Rβ), and common cytokine receptor gamma chain (γ_c) (*6*–*10*). Isolated IL-2Rα has been termed the "low-affinity" IL-2 receptor (binding affinity $K_d \approx$ 10 nM) and is not involved in signal transduction (*11*). A complex of IL-2Rβ and γ_c binds with intermediate affinity ($K_d \approx 1$ nM) and is the receptor form on NK cells, macrophages, and resting T cells (*2*), although IL-2Rβ alone has very low affinity ($K_d \approx 100$ nM) and γ_c alone has no detectable binding affinity for IL-2 (*12*). The association of IL-2Rβ and γ_c in the presence of IL-2 is necessary and sufficient for effective signal transduction through the heterodimerization of their cytoplasmic domains and subsequent kinase activation of multiple signaling pathways (*13*, *14*). A complex with three subunits—IL-2Rα, IL-2β, and γ_c—binds with high affinity ($K_d \approx$ 10 pM) and is the receptor form on activated T cells (*10*). The high-affinity receptor complex mediates most biological effects of IL-2 in vivo (*2*).

Whereas IL-2Rα is a specific receptor for IL-2, IL-2Rβ is also a component of the IL-15 receptor and γ_c is shared by cytokines IL-4, IL-7, IL-9, IL-15, and IL-21 (*15*). Mutations in γ_c can abolish the activity of all γ_c-dependent cytokines and result in X-linked severe combined immunodeficiency diseases (X-SCID), in which the T and NK cells are absent or profoundly reduced in number (*16*). Because the six γ_c-dependent cytokines have low sequence homology, structural information will be helpful to delineate shared versus ligand-specific binding determinants that could be exploited therapeutically. Previously, we reported the structure of the binary complex of IL-2 with IL-2Rα (*17*). We now present the crystal structure, at 2.3 Å resolution, of the quaternary complex of IL-2 with the extracellular domains of receptors IL-2Rα, IL-2Rβ, and γ_c.

Overall structure. Because of the heterogeneity of the fully glycosylated proteins expressed from insect cells, we crystallized a glycan-minimized quaternary complex, which had five potential Asn-linked glycosylation sites mutated (*18*). This material behaved identically to the fully glycosylated proteins and yielded crystals that diffracted to 2.3 Å resolution (*18*).

The quaternary complex is composed of one copy each of IL-2, IL-2Rα, IL-2Rβ, and γ_c (Fig. 1A). The orientation of IL-2Rα explains the necessity for the long connecting peptide, disordered in the structure, between the IL-2Rα globular head and the transmembrane segment. This allows the IL-2Rα binding domain to extend away from the cell surface and reach the dorsally located binding site on IL-2 (Fig. 1B). The bases of the receptors IL-2Rβ and γ_c, both class I–type cytokine receptors, converge to form a Y shape and IL-2 binds in the fork (Fig. 1, A and B). Formation of the quaternary complex is mediated by four binding sites—IL-2/IL-2Rα, IL-2/IL-2Rβ, IL-2/γ_c, and IL-2Rβ/γ_c—burying a total of 5700 Å^2 of surface area (fig. S1). The IL-2/IL-2Rα and IL-2/IL-2Rβ contacts are independent, whereas IL-2 and IL-2Rβ form a composite interface with γ_c, reflecting the cooperative nature of complex assembly.

IL-2Rα has been shown to deviate from typical cytokine receptor structure and mode of interaction with IL-2 (*17*). It is composed of two domain-swapped "sushi" modules, essentially miniature β-sheet sandwich domains. IL-2Rβ and γ_c are prototypical members of the class I cytokine receptor superfamily (*19*). Both are composed of N- and C-terminal fibronectin-III domains (D1 and D2, respectively), which are characterized by a β-sandwich sheet consisting of seven antiparallel strands arranged in a three-on-four topology. In IL-2Rβ, the D1 and D2 domains are connected by a helical linker and are bent at ~90°, whereas in γ_c the D1 and D2 domains are bent at ~120° (Fig. 1A). Both IL-2Rβ and γ_c contain the two disulfide bonds in the N-terminal domain (D1) and a "WSXWS" motif (*20*) in the C-terminal domain (D2) that are characteristic of class I cytokine receptors (*19*). However, a third disulfide bond in the γ_c D2 domain is unusual because of its central position in the interface with IL-2 and its role in enabling degenerate cytokine recognition (Cys^{160} to Cys^{209}) (discussed below).

IL-2/IL-2Rα. In the "low-affinity" complex, the atomic interactions between IL-2 and IL-2Rα, now visualized at 2.3 Å, are unchanged from the binary complex at 2.8 Å (*17*). The binding interface between IL-2 and IL-2Rα in the quaternary complex is composed of helices A′ and B′ and part of the AB loop in IL-2 and strands G, C, and D in the D1 domain and strand A in the D2 domain in IL-2Rα (table S2A). The two prominent hydrophobic ridges around residues Phe^{42} and Tyr^{45} of IL-2 insert into grooves between the IL-2Rα beta strands. Superposition of the two IL-2Rα structures in the binary and quaternary complexes shows a significant shift in the D2 domain of IL-2Rα (~2 Å), which is most likely a result of crystal packing and reflects some flexibility in the D1-D2 junction.

IL-15 is the only other cytokine that uses an atypical sushi-domain alpha receptor (IL-

Howard Hughes Medical Institute, Department of Microbiology and Immunology, and Department of Structural Biology, Stanford University School of Medicine, 299 Campus Drive, Fairchild D319, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: kcgarcia@stanford.edu

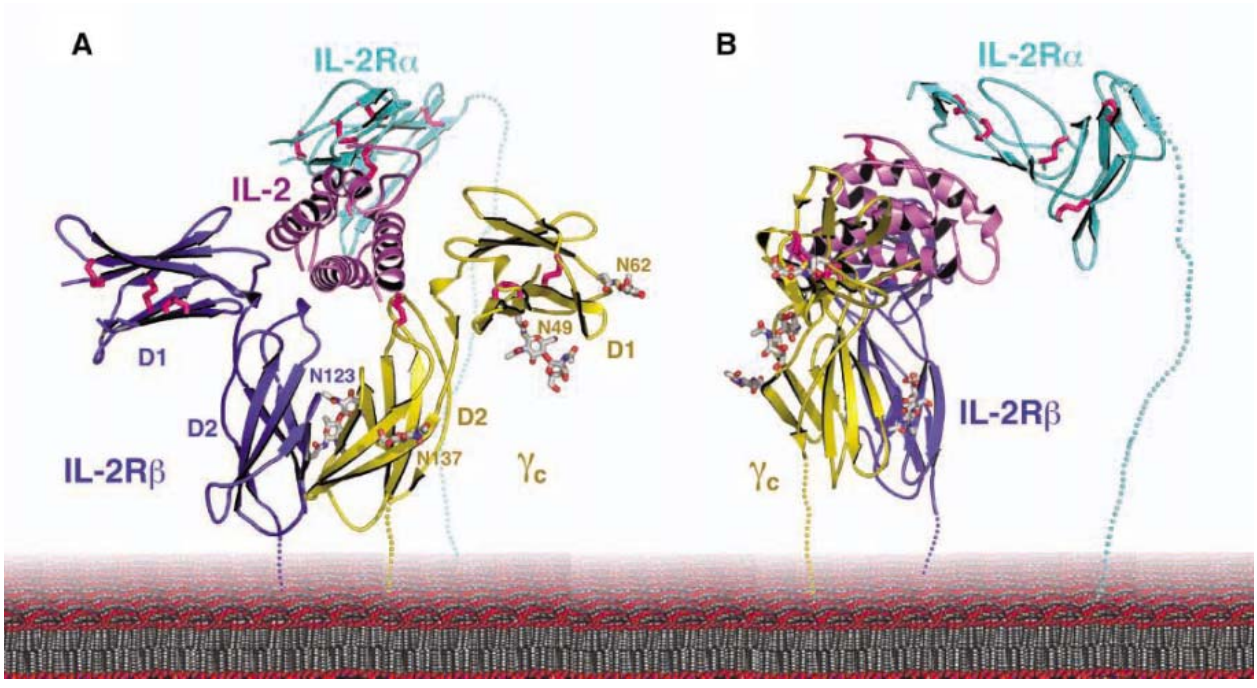


Fig. 1. Structure of the human IL-2/Rαβγ quaternary signaling complex. (**A** and **B**) Ribbon diagram of the complex structure shown in two views related by a ~90° rotation about the vertical axis. IL-2 is shown in violet, and the receptors are shown in cyan (IL-2Rα), blue (IL-2Rβ), and gold (γ_c). The observed N-linked carbohydrates at Asn^{123} of IL-2Rβ and at Asn^{49}, Asn^{62}, and Asn^{137} of γ_c are shown in gray (*20*). Disulfide bonds are shown in red. The disordered peptides connecting the C terminus of the receptors to the cell membrane are shown as dotted lines in their respective colors. The program PyMol (*43*) was used to make all figures.

15Rα), which is expressed primarily on NK cells (*21*). However, IL-15Rα is only a single sushi domain analogous to the IL-2Rα D1 domain (*22*, *23*). By analogy with IL-2, the IL-15/IL-15Rα complex likely forms first, followed by binding to IL-2Rβ and γ_c to form the quaternary signaling complex.

IL-2Rα does not appear to make any contact with either IL-2Rβ or γ_c. This is rather surprising, given that the IL-2/IL-2Rα complex binds with much higher affinity to IL-2Rβ ($K_d \approx 30$ pM) than does IL-2 binding to IL-2Rβ alone ($K_d \approx 100$ nM) (*12*, *24*) and the on-rate of IL-2 for IL-2Rβ is faster in the presence of IL-2Rα by a factor of 3 to 20 (*11*, *25*). We see no evidence of a composite receptor binding surface for IL-2, so what is the basis of the cooperativity? One possibility would be simple entropy reduction, wherein IL-2Rα captures and concentrates free IL-2 at the cell surface for presentation to IL-2Rβ and γ_c. Another possibility would be an IL-2Rα–induced conformational change in IL-2 that stabilizes the formation of the ternary complex.

To address the latter mechanism, we compared IL-2 structures in the quaternary complex, binary complex, and unbound states. The root mean square deviations for Cα atoms in the helical core between the different IL-2 molecules indicate nearly identical structures, ranging from 0.29 Å to 0.57 Å. One notable exception is at the beginning of helix C of IL-2, where, in the binary and quaternary complexes, several turns of helix C are slightly unwound and translated forward by ~1.0 Å toward IL-2Rβ (Fig. 2A). This local conformational adjustment moves IL-2 residue Asn^{88} into hydrogen-bonding distance to IL-2Rβ residue Arg^{42} (Fig. 2B). This movement possibly "primes" the next step in complex assembly by forming a more complementary IL-2Rβ binding site. Consistent with this, mutation of Asn^{88} in IL-2 ablates binding to IL-2Rβ (*5*). IL-2Rα may stabilize a favorable IL-2Rβ–binding conformation of IL-2 helix C, reducing a conformational entropy penalty that would be incurred during binding to IL-2Rβ. This priming of a "quiescent" IL-2Rβ binding site in IL-2 by IL-2Rα could effectively increase the on-rate for the IL-2 interaction with IL-2Rβ, as has been observed.

IL-2/IL-2Rβ. The interface between IL-2 and IL-2Rβ buries ~1350 Å^2 formed by residues from helices A and C in IL-2 and residues from loops CC′1, EF1, BC2, and FG2 in IL-2Rβ (table S2B). The interface is highly polar, with eight hydrogen bonds directly between IL-2 and IL-2Rβ residues. Strikingly, there are seven water molecules buried in the interface that bridge interactions between IL-2 and IL-2Rβ by forming bonds with protein atoms (Fig. 3A) (table S2B). Solvent exchange with the layer of water molecules between IL-2Rβ and IL-2 could explain the fast on- and off-rates and the weak affinity of the IL-2/IL-2Rβ binary complex. Two residues of IL-2 that have been shown by mutagenesis to be critical for IL-2Rβ binding, Asp^{20} and Asn^{88}, are involved in hydrogen bonding networks to both water molecules and side chains on IL-2Rβ (Fig. 3A). The side chains of IL-2Rβ residues His^{133} and Tyr^{134} insert into a complementary cavity in IL-2 to form hydrogen and ionic bonds with Asp^{20} of IL-2 (Fig. 3B).

IL-2Rβ is also used by IL-15 to form a quaternary complex along with IL-15Rα and γ_c (*15*). IL-15 has limited sequence identity (19%) with IL-2, so its contact with IL-2Rβ is probably through a unique set of interactions. The bridging water molecules may contribute to the ability of IL-2Rβ to cross-react by accommodating the different IL-15 residues through remodeling of the intervening solvent layer.

IL-2/γ_c. Neither IL-2 nor IL-2Rβ alone have measurable affinities for γ_c (*12*). Therefore, two very weak interactions, IL-2/γ_c and IL-2Rβ/γ_c, combine to produce an intermediate-affinity IL-2/IL-2Rβ/γ_c complex. In the quaternary complex structure, the interaction surface of the IL-2/Rαβ complex with γ_c is composed of two interfaces: a small one between IL-2 and γ_c, and a larger one between IL-2Rβ and γ_c.

The IL-2/γ_c interface buries ~970 Å^2 of surface area and is the smallest of the four protein-protein interfaces in the complex. The γ_c binding surface is striking in its absence of extended side chain–specific interactions with

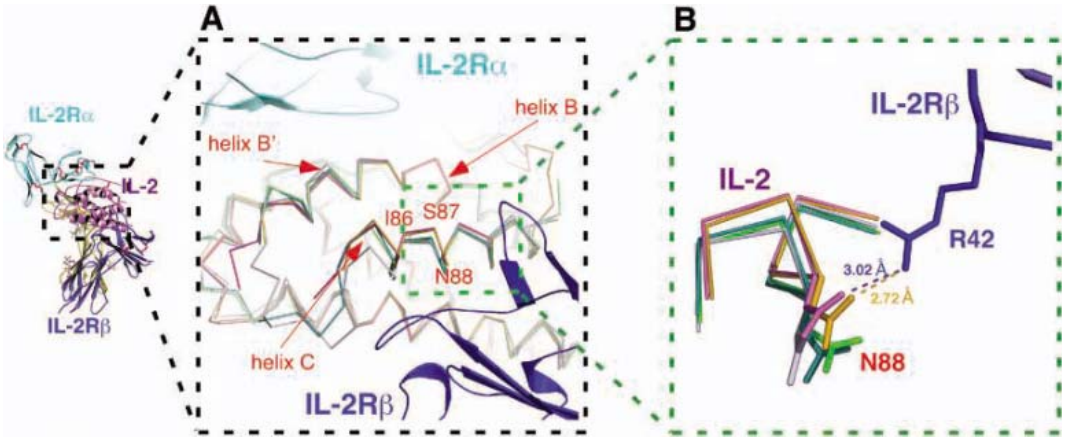


Fig. 2. IL-2Rα binding results in local conformational changes within IL-2 helix C. (**A**) Backbone superposition of IL-2 structures in quaternary complex (violet), binary complex (orange) (PDB 1Z92) (*17*), and three unbounded states: PDB 1M4C (green), 1M47 (dark green), and 3INK (gray) (*44*, *45*). (**B**) IL-2 residue Asn^{88} in helix C forms a hydrogen bond with Arg^{42} from IL-2Rβ in quaternary and binary complexes as a result of closer proximity induced by IL-2Rα binding.

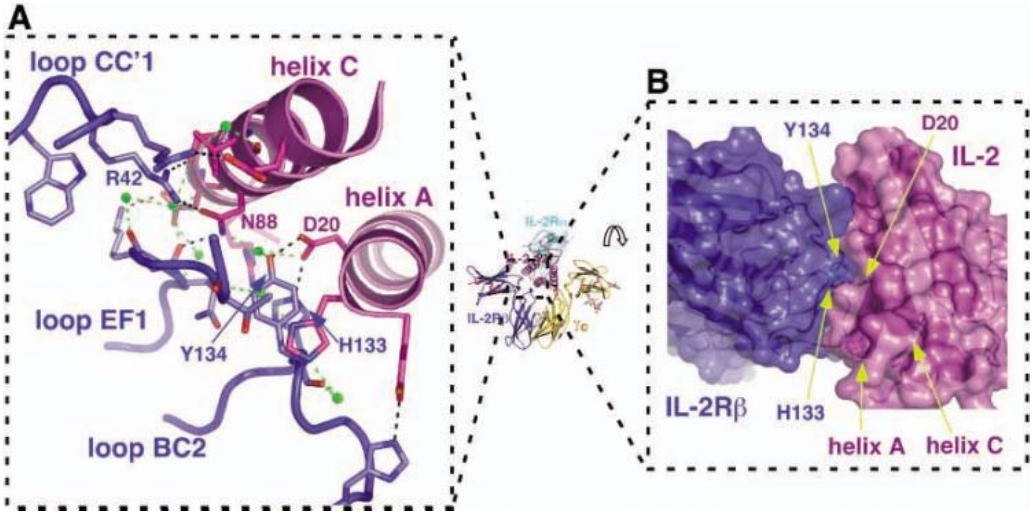


Fig. 3. A polar interface and hydration layer between IL-2 and IL-2Rβ. (**A**) All interactions between IL-2 and IL-2Rβ. The buried water molecules in the interface are shown as green spheres. The hydrogen bonds between IL-2 and IL-2Rβ are in black; those between water molecules and protein atoms are in green. (**B**) Close-up view of the shape complementarity in the interface, as viewed from above.

IL-2 and in the preponderance of main-chain contacts. Although there are several apparent ''hotspots,'' the γ_c binding surface is remarkable in its flatness and almost tangential contact with IL-2 (Fig. 4A). The γ_c structure contains an unusual disulfide bond in the heart of the interface with IL-2 that connects loops FG2 with BC2 and supports the conformations of Ser^{207} to Pro^{211} that form direct contacts with IL-2 (Fig. 4A). The disulfide also contributes to the apparent rigidity of the cytokine-binding surface, which is surprising given that one prevailing assumption for receptor cross-reactivity is structural plasticity (*26*). The γ_c binding surface does not appear to contain mobile structural elements, although we do not have a structure of the unliganded receptor for comparison.

The overall interface involves residues from helices A and D in IL-2 and residues from loops CC′1, EF1, BC2, FG2, and the linker between strands G1 and A2 in γ_c (table S2C). In contrast to the broad array of specific polar interactions between IL-2 and IL-2Rβ, small contact patches dominate the IL-2/γ_c interface. The first one is composed of residue Tyr^{103} from γ_c and residues Ser^{127} and Ser^{130} from IL-2. The Tyr^{103} aromatic ring packs flat against the side chains of Ser^{127} and Ser^{130} in IL-2 (Fig. 4, A and B). The second is around residue Gln^{126} in IL-2, which has been shown by mutagenesis to be a critical energetic hotspot. Similar to Tyr^{103}, the side chain of Gln^{126} is almost parallel to the surface formed by main-chain atoms of residues Pro^{207} to Ser^{211} in γ_c, and this orientation is further fixed by two hydrogen bonds with the receptor, to Pro^{207} O and Ser^{211} OG (Fig. 4A). A single bridged water molecule in the IL-2/γ_c interface forms hydrogen bonds with Gln^{126} of IL-2 and with Gln^{127} and Asn^{128} of γ_c, respectively (table S2C).

Previous mutagenesis studies have found that two of the flat γ_c patches we see in the structure that are involved in binding IL-2—residue Tyr^{103} and residues from Leu^{208} to Ser^{211} in γ_c—are also important for binding IL-4, IL-7, IL-15, and IL-21 (*27*–*29*). There is also evidence that γ_c binding sites for different cytokines overlap but are not identical (*30*). We propose that these two patches form the central degenerate recognition surfaces that participate in binding all cytokines in the γ_c-dependent family by using their flat surfaces, and that the peripheral polar interactions modulate specificity for individual cytokines.

IL-2Rβ/γ_c. The second part of the composite interface between IL-2/Rβ and γ_c is formed by extensive interactions between the D2 domains of IL-2Rβ and γ_c, burying more than 1750 $Å^2$ of surface area (Fig. 5A). The D2 domains from IL-2Rβ and γ_c are related by almost exact two-fold symmetry, and the interface is formed by 21 residues from IL-2Rβ and 19 residues from γ_c from strands C2, C′2, and E2 and loop C′E2 (table S2D). The interface is highly polar, with a peripheral ring of 17 hydrogen bonds surrounding a hydrophobic stripe in the center dominated by Trp^{166} from IL-2Rβ and Tyr^{167} from γ_c (Fig. 5B) (table S2D).

The D2-D2 interaction between IL-2Rβ and γ_c is the largest buried surface seen so far in cytokine-receptor complexes, and it underscores the role of receptor-receptor contact in the cooperative assembly of the quaternary complex. Although it is surprising that IL-2Rβ and γ_c have no measurable affinity toward one another given this extensive contact surface, a lack of interaction would prevent the receptors from heterodi-

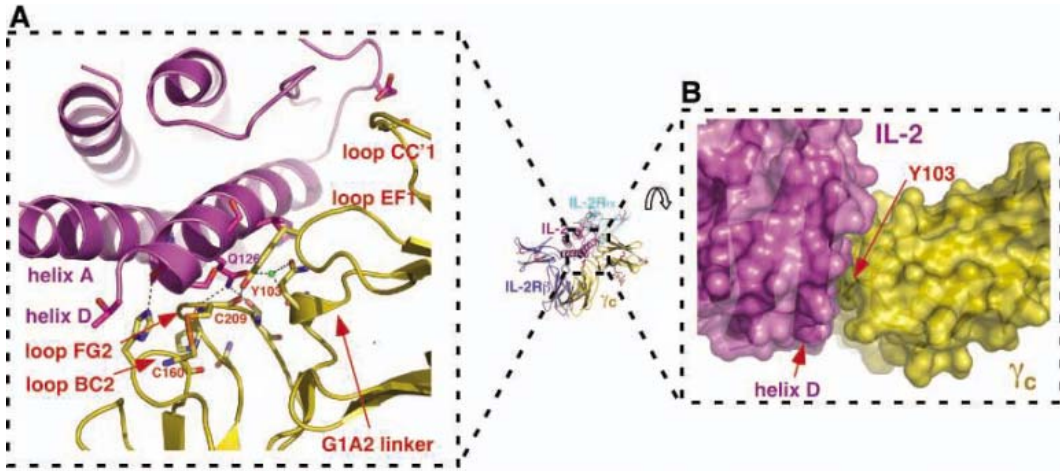


Fig. 4. Interactions between γ_c and IL-2. (**A**) Contacting residues in the IL-2/γ_c interface. (**B**) Surface representation of the relative contact patch around Tyr[103] from γ_c, as viewed from above.

merizing and signaling in the absence of cytokine. Given the structural observations of a small IL-2/γ_c interface and a large and tightly packed IL-2Rβ/γ_c interface, we suggest that the receptor-receptor (i.e., D2-D2) contact may serve as an important energetic determinant. If so, the role of the cytokine would be to stabilize complex formation by guiding a perfect geometrical alignment of the numerous interatomic contacts (hydrogen bonds, van der Waals contacts, etc.) in the D2-D2 interface. In this respect, considering the relatively flat and chemically inert IL-2/γ_c interface, it may be that specificity is largely provided by receptor-receptor contact with IL-2Rβ rather than cytokine.

This model can in part be rationalized by the ''shared'' function of γ_c. γ_c is expressed on most immune cell types, but the tissue and cytokine specificity are regulated by the coordinate expression of different α receptors (or, in the case of IL-2, the β receptor). Given the lack of sequence homology between γ_c-dependent cytokines, the capacity of γ_c to discriminate among (and to cross-react with) these ligands would be more easily achieved by spreading the energetics of the interaction over the combined ~2600 Å^2 of surface area presented by the IL-2/IL-2Rβ composite surface, rather than focusing it all on the small portion of this surface contributed by cytokine alone (~970 Å^2). By comparison, in the structures of more ligand-specific cytokine receptors such as human growth hormone receptor (hGHbp) and erythropoietin receptor (epoR) complexed with their ligands, there is much less receptor-receptor contact (~900 Å^2 for hGHbp, no contact for epoR) (*31*, *32*).

X-SCID mutations. X-linked severe combined immunodeficiency (SCID) is a syndrome of profoundly impaired cellular and humoral immunity caused by mutations in the gene encoding the common gamma chain (*33*). The mutated gene results in faulty signaling through several cytokine receptors; thus, T, B, and NK cells can be affected by a single mutation. We mapped extracellular γ_c mutations that have been found in X-SCID patients in which γ_c is expressed but is not competent for activation by any of the γ_c cytokines. Many mutations appear to concentrate near the γ_c cytokine-binding site, and several of these—Y103N, Y103C, L208P, C209R, C209Y, G210R, G210V, and C160R (*20*, *33*)—map to the γ_c binding interface with IL-2 (Fig. 6A). Mutation of Cys[209] or Cys[160], which participate in the disulfide bond in the γ_c cytokine-binding surface, would be particularly destabilizing. The interface mutations would effectively ablate cytokine recognition by γ_c, but it seems likely that the receptor would still appear to be competent to signal if heterodimerized. Although the database X-SCID mutations map to all other parts of the γ_c structure, none of the X-SCID mutations map to the IL-2Rβ/γ_c interface, possibly implying a structural necessity for this area to be preserved in the expressed receptor (Fig. 6B).

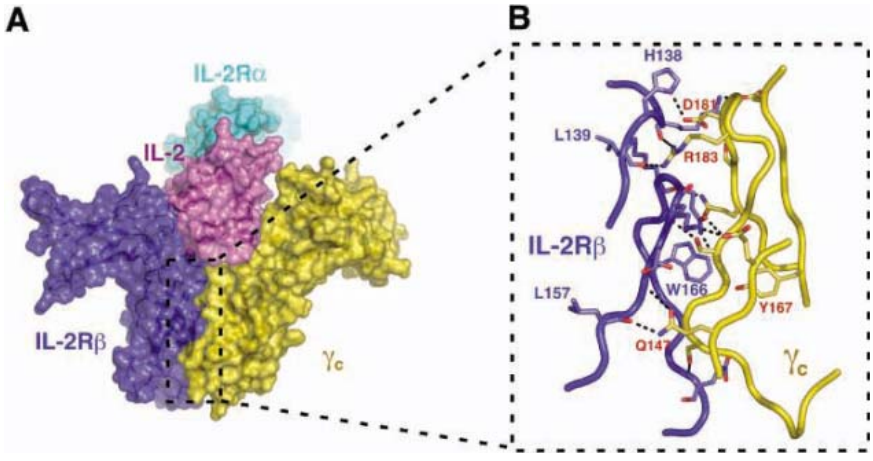


Fig. 5. Extensive receptor-receptor contact between IL-2Rβ and γ_c. (**A**) Surface representation of the quaternary complex shows the shape complementarity in the IL-2Rβ/γ_c interface. (**B**) Hydrogen-bonding interactions between IL-2Rβ and γ_c.

Degenerate cytokine recognition by γ_c. The γ_c-dependent cytokines have, on average, 19% sequence identity to one another, with most of the homology concentrated inside the helical cores. Although we currently know the structures of only IL-2 and IL-4 in the γ_c-dependent cytokine family, we sought to identify conserved residues that might serve as a recognition code for γ_c binding throughout the family. Sequence alignment between IL-2 and other γ_c-dependent cytokines (fig. S3) indicates that residue Gln[126], which plays a key structural role in IL-2 interactions with γ_c, is conserved in IL-2, IL-9, IL-15, and IL-21, whereas IL-4 and IL-7 have Arg[121] and Lys[139] in this position, respectively. Superposition of the IL-4/IL-4Rα complex (*34*) with the IL-2 quaternary complex indicates that Arg[121] may play a structural role similar to that of Gln[126] in IL-2 in contacting γ_c. Although position 126 in helix D may serve as a common contact point with γ_c, there are not obvious constellations of conserved residues that allow us to dock the different cytokines with γ_c. It appears that each cytokine uses distinct structural solutions for γ_c recognition.

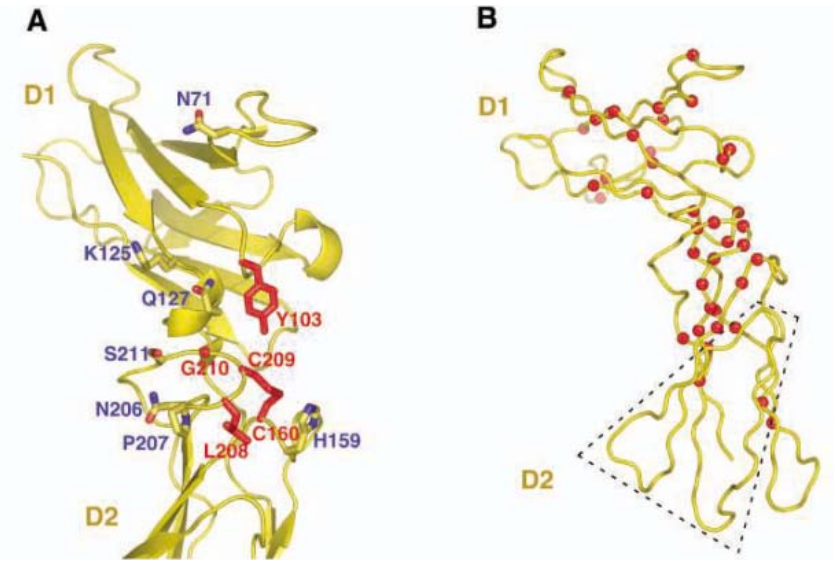


Fig. 6. Mapping known X-SCID mutations in the structure of γ_c. (**A**) Five missense mutations that are located in the γ_c cytokine-binding epitope, and make contact with IL-2 in the structure, are shown as red sticks. (**B**) Distribution of all missense mutations in the X-SCID mutation database (http://genome.nhgri.nih.gov/scid) in γ_c. The γ_c area participating in the D2-D2 interaction with IL-2Rβ is free of mutations and is indicated within the dashed line.

Cytokine recognition by shared receptors. The flat and apparently rigid surface in the common binding epitope of γ_c suggests that it uses somewhat chemically inert complementary surfaces to interact with divergent cytokine residues. Although this contrasts with notions of receptor promiscuity through binding site flexibility (*26*), it parallels structural results for gp130, the shared cytokine receptor for long-chain cytokines (*35*), in complex with three different cytokines: LIF, viral IL-6, and human IL-6 (*36*–*38*). In the gp130 system, thermodynamic compensation between rigid surfaces, rather than conformational change, enables cross-reactivity with a broad range of chemically diverse cytokine surfaces (*35*). We predict, on the basis of direct thermodynamic measurements of the quaternary complex assembly (*12*), that γ_c also uses such a mechanism for cross-reactivity. Such a large range of energetic compensation appears to be a property of binding sites found in shared receptors, which are tuned for degenerate recognition through a mechanism that bypasses the entropic penalty for conformational change (*39*).

Therapeutic implications. A recombinant human IL-2 (rIL-2) analog (Aldesleukin, Proleukin, Chiron Inc., Emeryville, CA) is currently licensed in the United States for the treatment of metastatic melanoma and renal cell carcinoma and is undergoing clinical trials for patients with HIV/AIDS (*40*, *41*). Treatment of cancer patients with rIL-2 results in robust responses but is associated with life-threatening toxicity, which limits its use (*40*). The antitumor efficacy of rIL-2 therapy has been shown to be mediated by the high-affinity quaternary complex containing IL-2Rαβγ expressed on T cells, whereas the toxic side effects are mediated through the IL-2Rβγ form of the receptor on NK cells (*42*). This hypothesis suggests that it might be possible to dissociate efficacy and toxicity by generating an IL-2 variant with selectivity for the IL-2Rαβγ receptor complex, versus the IL-2Rβγ complex of NK cells (*5*). Proof-of-concept was demonstrated with an IL-2 variant bearing an $Asn^{88} \rightarrow Arg$ mutation that conferred a factor of 3000 selectivity increase for the IL-2Rαβγ complex by crippling the interaction between IL-2 and IL-2Rβ (*5*).

In the structure we see that Asn^{88} is the side chain brought into hydrogen-bonding distance to IL-2Rβ by the structural perturbation of helix C in response to IL-2Rα binding, and is involved in an extensive hydrogen-bonding network (Fig. 3A). Such an energetically critical residue may not be the most desirable choice for generating a receptor-selective IL-2, because it may not be necessary to completely ablate binding to the IL-2Rβγ receptors. Rather, weakening the IL-2Rβ interaction, or even contact with γ_c, while maintaining near wild-type affinity for the IL-2Rαβγ complex appears tenable through structure-guided engineering. It is our hope that this quaternary complex structure can be used to design IL-2 variants that will allow its powerful clinical potential to be more fully realized.

References and Notes

1. K. A. Smith, *Science* **240**, 1169 (1988).
2. B. H. Nelson, D. M. Willerford, *Adv. Immunol.* **70**, 1 (1998).
3. J. Theze, P. M. Alzari, J. Bertoglio, *Immunol. Today* **17**, 481 (1996).
4. A. C. Church, *Q JM* **96**, 91 (2003).
5. A. B. Shanafelt *et al.*, *Nat. Biotechnol.* **18**, 1197 (2000).
6. W. J. Leonard *et al.*, *Nature* **311**, 626 (1984).
7. T. Nikaido *et al.*, *Nature* **311**, 631 (1984).
8. D. Cosman *et al.*, *Nature* **312**, 768 (1984).
9. M. Hatakeyama *et al.*, *Science* **244**, 551 (1989).
10. T. Takeshita *et al.*, *Science* **257**, 379 (1992).
11. H. M. Wang, K. A. Smith, *J. Exp. Med.* **166**, 1055 (1987).
12. M. Rickert, M. J. Boulanger, N. Goriatcheva, K. C. Garcia, *J. Mol. Biol.* **339**, 1115 (2004).
13. Y. Nakamura *et al.*, *Nature* **369**, 330 (1994).
14. B. H. Nelson, J. D. Lord, P. D. Greenberg, *Nature* **369**, 333 (1994).
15. K. Ozaki, W. J. Leonard, *J. Biol. Chem.* **277**, 29355 (2002).
16. W. J. Leonard, *Nat. Rev. Immunol.* **1**, 200 (2001).
17. M. Rickert, X. Wang, M. J. Boulanger, N. Goriatcheva, K. C. Garcia, *Science* **308**, 1477 (2005).
18. See supporting data on *Science* Online.
19. J. F. Bazan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6934 (1990).
20. Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
21. B. Becknell, M. A. Caligiuri, *Adv. Immunol.* **86**, 209 (2005).
22. D. M. Anderson *et al.*, *J. Biol. Chem.* **270**, 29862 (1995).
23. J. G. Giri *et al.*, *EMBO J.* **14**, 3654 (1995).
24. N. Arima *et al.*, *J. Exp. Med.* **176**, 1265 (1992).
25. S. F. Liparoto, T. L. Ciardelli, *J. Mol. Recognit.* **12**, 316 (1999).
26. S. Atwell, M. Ultsch, A. M. De Vos, J. A. Wells, *Science* **278**, 1125 (1997).
27. F. Olosz, T. R. Malek, *J. Biol. Chem.* **277**, 12047 (2002).
28. J. L. Zhang, M. Buehner, W. Sebald, *Eur. J. Biochem.* **269**, 1490 (2002).
29. J. L. Zhang, D. Foster, W. Sebald, *Biochem. Biophys. Res. Commun.* **300**, 291 (2003).
30. N. Raskin *et al.*, *J. Immunol.* **161**, 3474 (1998).
31. O. Livnah *et al.*, *Science* **273**, 464 (1996).
32. R. S. Syed *et al.*, *Nature* **395**, 511 (1998).
33. J. M. Puck, *Immunol. Today* **17**, 507 (1996).
34. T. Hage, W. Sebald, P. Reinemer, *Cell* **97**, 271 (1999).
35. M. J. Boulanger, K. C. Garcia, *Adv. Protein Chem.* **68**, 107 (2004).
36. M. J. Boulanger, A. J. Bankovich, T. Kortemme, D. Baker, K. C. Garcia, *Mol. Cell* **12**, 577 (2003).
37. D. Chow, X. He, A. L. Snow, S. Rose-John, K. C. Garcia, *Science* **291**, 2150 (2001).
38. M. J. Boulanger, D. C. Chow, E. E. Brevnova, K. C. Garcia, *Science* **300**, 2101 (2003).
39. B. J. McFarland, R. K. Strong, *Immunity* **19**, 803 (2003).
40. J. Dutcher, *Oncology (Huntingt.)* **16**, 4 (2002).
41. K. A. Smith, B. A. Boyle, *AIDS Read.* **13**, 365 (2003).
42. K. A. Smith, *Blood* **81**, 1414 (1993).
43. W. L. DeLano, *The PyMOL Molecular Graphics System* (DeLano Scientific, San Carlos, CA, 2002).
44. M. R. Arkin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1603 (2003).
45. D. B. McKay, *Science* **257**, 412 (1992).

46. We thank N. Goriatcheva for expert technical assistance; K. Smith, T. Springer, and W. Leonard for helpful discussions; and the Advanced Light Source (UC-Berkeley) and Stanford Synchrotron Radiation Laboratory for synchrotron beamtime. Supported by the Keck Foundation, HHMI, and NIH grant AI51321. Coordinates and structure factors have been deposited in the Protein Data Bank with accession number 2B5I.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1159/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References

25 July 2005; accepted 14 October 2005
10.1126/science.1117893

Thermodynamics of an Incommensurate Quantum Crystal

P. W. Anderson, W. F. Brinkman, David A. Huse*

We present a theory of the thermodynamics of an incommensurate quantum solid. The ground state of the solid is assumed to be an incommensurate crystal, with quantum zero-point vacancies and interstitials and thus a non-integer number of atoms per unit cell. We show that at low temperature T, the variation of the net vacancy concentration should be as T^4 and that the first correction to the specific heat due to this varies as T^7; these are quite consistent with experiments on solid helium-4. We also make some observations about the recent experimental reports of "supersolidity" in solid helium-4 that motivate a renewed interest in quantum crystals.

Recent experiments (*1*, *2*) showing a marked low temperature reduction in the rotational moment of inertia of crystals of solid ^{4}He have rekindled interest in this highly quantum-mechanical solid. The proposed "supersolid" phase is believed to occur as a result of the quantum behavior of point defects, namely vacancies and interstitials, in this crystal of bosons (*3*). Over the past 20 years, various experiments have been performed to measure the temperature dependence of the vacancy concentration in solid ^{4}He. Although there are considerable differences in the results, the most accurate data comes from x-ray measurements of the lattice constant as a function of temperature at fixed density (*4*). These data have usually been interpreted in terms of a classical theory of vacancies involving an activation energy and a configurational entropy for their creation. However, this theory implies a corresponding vacancy contribution to the specific heat that is as large as the phonon contribution near 1 K (*5*). Such a classical vacancy contribution to the specific heat has not been seen; the specific heat is instead well explained almost entirely in terms of the T^3 term from the phonon spectrum, and the leading correction to this fits very well to a T^7 term (*6*). There have been various attempts to explain this discrepancy, but none have been satisfactory and the problem has remained open (*5*).

Here, we propose a simple phenomenological thermodynamic description of a low-temperature incommensurate quantum solid. We note that the ground state of a quantum solid need not be commensurate, i.e., it need not have an integer number of atoms per unit cell (*3*). One description of the quantum solid is as a density wave that has formed in the quantum fluid. The periodicity of this density wave need not match precisely to the particle density, so that the ground state may be incommensurate, with unequal densities of vacancies and interstitials. The x-ray measurements on solid hexagonal-close-packed (hcp) ^{4}He show that the density of vacancies increases faster than that of interstitials with increasing temperature (*4*), indicating that thermal fluctuations favor vacancies more than interstitials. Whether or not the same is true for quantum fluctuations is not clear at this point. We develop a simple thermodynamic theory of the low temperature behavior of an incommensurate quantum solid, finding that the low temperature net change in vacancy density at fixed particle density follows a T^4 power law behavior. The x-ray data are quite consistent with such a temperature dependence, as we show below. In addition, we show that this simple model produces a T^7 correction to the specific heat, as has been observed (*6*). Such a scenario could apply to any highly quantum solid and is not specific to bosons, so solid ^{3}He should and does show similar phenomena (*7*, *8*); perhaps hydrogen might, also.

It has been argued by one of us based on Jastrow-type wave functions that it is expected that there will be vacancies in the ground state of a highly quantum fluctuating solid such as ^{4}He and that such a ground state may be superfluid (*9*). The vacancies are an integral feature of the ground state and carry no entropy or energy. These vacancies may be sufficiently mobile that they never behave as classical particle-like objects at the temperatures where the solid is present. Thus, we will assume that the vacancies and interstitials in solid ^{4}He remain in a strongly correlated quantum state up to temperatures in the vicinity of 1 Kelvin, so they do not make a large contribution to the specific heat other than the incommensurability effect that we describe below.

For an hcp lattice of volume V and lattice constant a, the number of lattice sites is $N_s = V\sqrt{2}/a^3$. If the ground state crystal is incommensurate, then its number N of atoms differs from its number of sites: $N \neq N_s$. Recent data on the possible superfluid nature of these solids (*2*) suggests that these two numbers could differ by up to 1%, although it seems quite possible that the 1% effect in the apparent superfluid density could arise from a much smaller (or even zero) net density of defects. Such a small difference between the number of atoms and the number of lattice sites may have escaped detection in simulations (*10*–*12*) of the ground state of solid ^{4}He. Direct comparisons of experimental measurements of the density of ^{4}He atoms to the x-ray density of sites do not appear to have been published for the low-pressure hcp phase where the apparent supersolidity has been seen, although Simmons (*13*) tells us that the difference appears to be well under 1%. We thus strongly urge that more simultaneously precise density and lattice constant measurements be done for the quantum solids to learn how incommensurate their ground states really are, especially at the lowest densities, where quantum fluctuations should be strongest.

Given that a crystal may be incommensurate, one needs to develop a theory in which the lattice constant and the density can change independently. In the temperature range we consider, the vacancies and interstitials are assumed to be incorporated in a highly correlated quantum state of the system, and the only low frequency modes giving large contributions to the temperature dependence of the free energy are the phonons. In the standard treatment of the low-temperature thermal expansion of a crystal, it is the density dependence of the phonon velocities (the Gruneisen parameters) that determine the expansion. Here, we will instead work at fixed particle density but allow the lattice constant and thus the incommensurability to vary, driven by the dependence of the phonon velocities on the incommensurability. Thus, we consider the free energy for a given mass of helium at a fixed volume, so that we do not need to include the overall density as a variable. Let the incommensurability

$$\varepsilon = \frac{N_s - N}{N_s} = \varepsilon_0 + \delta \qquad (1)$$

be the net fractional vacancy number (i.e., the fraction of vacancies minus the fraction of interstitials). We will ask about the crystal's behavior as a function of its incommensurability, although this is not a variable that is under ready experimental control. Here, ε_0 is

Department of Physics, Princeton University, Princeton, NJ 08544, USA.

*To whom correspondence should be addressed. E-mail: huse@princeton.edu

the incommensurability at absolute zero temperature. Thus we obtain the following expression for the free energy as an expansion at low temperature and low deviation δ of the incommensurability from the ground state value

$$F = -E_0 + \frac{E_2}{2}\delta^2 - (D_0 + D_1\delta + \ldots)T^4 + \ldots \quad (2)$$

$-E_0$ is the ground state energy, and E_2 gives the harmonic increase of the crystal's energy when, staying at $T = 0$, the incommensurability is changed away from its ground state value by changing the number of lattice sites and thus the lattice constant. The T^4 term in the free energy is simply that due to acoustic phonons and possibly also the acoustic superfluid mode that is expected to be present in a supersolid. The velocities of these acoustic modes in general vary with the incommensurability and are not at an extremum at the ground state incommensurability (which is $\delta = 0$). Thus, there is a term that is linear in δ in the prefactor of this T^4 term, from its lowest order linear variation with the incommensurability ε. The parameter D_1 plays the role of the Gruneisen parameter in driving the change in the lattice constant with temperature, but here this change is happening at fixed particle density. Next, we find the value of the incommensurability that minimizes this free energy (Eq. 2) at a given low temperature, obtaining to lowest order the temperature dependence

$$\delta \approx \frac{D_1}{E_2}T^4 \quad (3)$$

instead of the classical thermally activated form [$\delta \sim \exp(-\Delta/k_BT)$] that one obtains in the classical vacancy theory. Figure 1 shows that the x-ray data (*4*) fits about as well to our proposed T^4 temperature dependence as it does to the classical theory. Clearly, when similar measurements are made more precisely and/or carried to lower temperatures, a discrimination between these two simple theories will be made; again, we encourage such efforts. Note that here δ is the increase in the fractional net density of vacancies above the possibly nonzero value it already has in the ground state. Also, the strong quantum fluctuations might mean that the ground state concentrations of vacancies and interstitials are both rather larger than ε_0, but it is only the difference between these densities (that we are calling the net density) that is readily measurable and that enters as a thermodynamic parameter.

If instead the ground state is commensurate ($\varepsilon_0 = 0$) and is locked in to a Mott "insulating" state with exactly one atom per lattice site, then the energy as a function of the change in the incommensurability δ is linear ($\sim |\delta|$) rather than quadratic. This results in the classical thermally activated behavior (it can be viewed as activation of atomic "carriers" across the Mott gap of this insulator). Another possibility is that the quantum fluctuations are strong enough to put the system out of the Mott insulating phase, but the ground state remains commensurate with $\varepsilon_0 = 0$ because of an approximate (one might say "coincidental") vacancy-interstitial symmetry of the ground state. In this latter case, the $\delta \sim T^4$ behavior will occur, provided the thermal excitations break that approximate symmetry, as they certainly appear to from the x-ray data (*4*) (Fig. 1).

A second known anomaly follows from Eq. 2. The specific heat of solid ^{4}He in the temperature range near 1 Kelvin was shown to fit nearly exactly [see figure 7 in (*6*)] to the sum of two power laws:

$$C = AT^3 + BT^7 \quad (4)$$

The phonons give corrections to the T^3 specific heat due to their anharmonicity and dispersion, but these are expected to be down from the leading T^3 by powers of (T/Θ_D), where the Debye temperature $\Theta_D \cong 25$ K for helium. The observed T^7 correction is orders of magnitude larger than this (*6*). Minimizing Eq. 2 with respect to δ, the free energy as a function of temperature behaves as

$$F = -E_0 - D_0T^4 - D_1^2T^8/2E_2 + \ldots \quad (5)$$

Thus, the incommensurate crystal shows a positive T^7 leading correction to the phonon specific heat, due to its change of incommensurability with temperature. This is quite consistent with the experimental specific heat measurement (*6*). The x-ray and specific heat experiments together give rough estimates of $E_2 \cong 80$ K/atom, $D_0 \cong 0.013$ (K^3-atom)$^{-1}$, and $D_1 \cong 0.06$ (K^3-atom)$^{-1}$ for the parameters in our free energy (taking $k_B = 1$). The new parameters E_2 and D_1 are of the same order as, but larger than, E_0 and D_0, respectively, all of which seems reasonable.

It should be noted that nowhere in the present argument did we invoke the boson nature of ^{4}He. In fact, the discrepancies found in ^{4}He between the temperature-dependent x-ray vacancy data and the specific heat data within a classical vacancy model are also there in solid ^{3}He (*7*). There are quantitative differences between the isotopes, however, in that the corrections to the leading T^3 in the specific heat are much larger in ^{3}He (*8*). In fact, for ^{3}He the correction to the leading T^3 term becomes larger than the T^3 term itself and does not fit well to a simple T^7 correction (*8*). However, when the correction is that large, it should be expected that terms beyond T^7 cannot be neglected. Of course, the difference between bosons and fermions is essential when considering supersolidity, but it is not crucial for the thermodynamic issues we have discussed above.

Before concluding, we make a few comments about the recent experimental indications (*2*) of "supersolid" behavior in solid ^{4}He. We note the strong dissipation feature seen in the amplitude of their oscillator versus temperature in figure 2A of (*2*). This dissipation should be significant only when the rate of damping of the superflow is of the same order as the frequency of the oscillator, which is about 1 kHz. The broad (on the temperature axis) dissipation feature implies that this damping rate is decreasing rather gradually with decreasing temperature and passes through 1 kHz near the maximum damping, around $T = 60$ mK. The appearance of a detectable, apparently supersolid, signal at much higher temperature should not be viewed as a possible supersolid phase transition at those higher temperatures but instead possibly as the temperature where the precursors to supersolidity (the critical fluctuations) become detectable in this experiment. This very broad regime with precursors to the apparent supersolidity suggests to us two possibilities: first, that perhaps these experiments are near a supersolid quantum critical point, where the quantum fluctuations destroy supersolid order

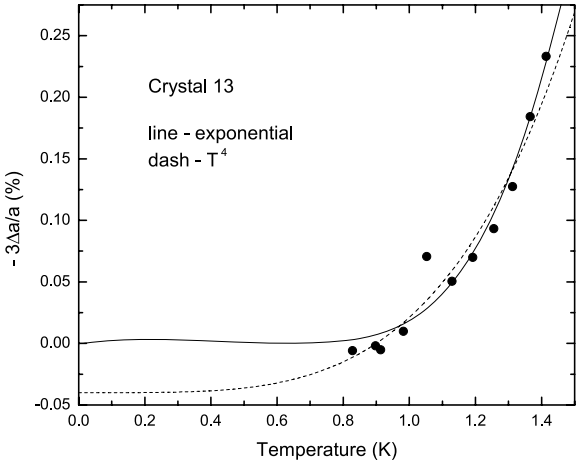


Fig. 1. The percentage net density of vacancies in a solid ^{4}He crystal of molar volume 20.9 cm^3, as measured using x-rays via the change Δa in the lattice constant from a reference value. Filled circles are the data from (*4*). The solid line is a thermally activated (classical) fit, whereas the dashed line is a fit to the $a \approx a_0 - bT^4$ behavior we expect if the ground state is incommensurate. The zero on the vertical axis is free and was chosen so that the classical fit goes to that value in the low T limit. (Figure courtesy of Ralph Simmons.)

in the ground state, replacing it with some sort of quantum vortex liquid ground state; or second, that the superflow is being damped by some temperature-dependent mechanism other than vortices (transverse phonons and *umklapp* are two possibilities that are not present in the liquid phase), and this damping only vanishes at zero temperature. Note that here we are always discussing the damping at linear order in the apparent superfluid velocity, thus in linear response to the solid's motion. The actual supersolid transition is where this rate of damping vanishes, so one can have a true superflow in linear response. From these recent experiments at just the one frequency (*2*), we cannot determine where this transition actually happens, or whether it does happen even at zero temperature, although we should conclude from their data that the supersolid transition temperature must be below the dissipation feature, which puts it below 50 mK (*14*). The results of similar experiments at other frequencies should be informative.

References and Notes

1. E. Kim, M. H. W. Chan, *Nature* **427**, 225 (2004).
2. E. Kim, M. H. W. Chan, *Science* **305**, 1941 (2004).
3. A. F. Andreev, I. M. Lifshitz, *JETP* **29**, 1107 (1969).
4. B. A. Fraass, P. R. Granfors, R. O. Simmons, *Phys. Rev. B* **39**, 124 (1989).
5. C. A. Burns, J. M. Goodkind, *J. Low Temp. Phys.* **95**, 695 (1994).
6. W. R. Gardner, J. K. Hoffer, N. E. Phillips, *Phys. Rev. A.* **7**, 1029 (1973).
7. S. M. Heald, D. R. Baer, R. O. Simmons, *Phys. Rev. B* **30**, 2531 (1984).
8. D. S. Greywall, *Phys. Rev. B* **15**, 2604 (1977).
9. P. W. Anderson, *arXiv*, cond-mat/0504731, 7 October 2005.
10. B. Chaudhuri, F. Pederiva, G. V. Chester, *Phys. Rev. B* **60**, 3271 (1999).
11. D. M. Ceperley, B. Bernu, *Phys. Rev. Lett.* **93**, 155303 (2004).
12. D. E. Galli, M. Rossi, L. Reatto, *Phys. Rev. B* **71**, 140506.
13. R. O. Simmons, private communication (2005).
14. In the qualitatively similar oscillator data for superfluid ^{4}He films near their Kosterlitz-Thouless transitions, the actual transition temperature is well below the dissipation feature (*15*).
15. D. J. Bishop, J. D. Reppy, *Phys. Rev. Lett.* **40**, 1727 (1978).

16. We thank M. Chan, R. Simmons, and D. Greywall for helpful discussions. This work was supported in part (D.A.H.) by NSF through grant DMR-0213706.

9 August 2005; accepted 13 October 2005
Published online 3 November 2005;
10.1126/science.1118625
Include this information when citing this paper.

Porous, Crystalline, Covalent Organic Frameworks

Adrien P. Côté,[1]* Annabelle I. Benin,[1] Nathan W. Ockwig,[1] Michael O'Keeffe,[2] Adam J. Matzger,[1] Omar M. Yaghi[1]*

Covalent organic frameworks (COFs) have been designed and successfully synthesized by condensation reactions of phenyl diboronic acid $\{C_6H_4[B(OH)_2]_2\}$ and hexahydroxytriphenylene $[C_{18}H_6(OH)_6]$. Powder x-ray diffraction studies of the highly crystalline products $(C_3H_2BO)_6\bullet(C_9H_{12})_1$ (COF-1) and $C_9H_4BO_2$ (COF-5) revealed expanded porous graphitic layers that are either staggered (COF-1, *P6$_3$/mmc*) or eclipsed (COF-5, *P6/mmm*). Their crystal structures are entirely held by strong bonds between B, C, and O atoms to form rigid porous architectures with pore sizes ranging from 7 to 27 angstroms. COF-1 and COF-5 exhibit high thermal stability (to temperatures up to 500° to 600°C), permanent porosity, and high surface areas (711 and 1590 square meters per gram, respectively).

The design and synthesis of crystalline extended organic structures in which the building blocks are linked by strong covalent bonds are undeveloped areas of research. It is widely believed that the required microscopic reversibility of the crystallization of linked organic molecules into such solids is difficult if not impossible to achieve (the crystallization problem). The lack of crystalline cross-linked polymers is often cited as evidence in support of this view (*1*). Recently, we embarked on a program aimed at challenging this notion by constructing porous, crystalline, covalent organic frameworks (COFs) solely from light elements (H, B, C, N, and O) that are known to form strong covalent bonds in well-established and useful materials such as diamond, graphite, and boron nitride. The successful realization of COF materials through molecular building blocks would provide covalent frameworks that could be functionalized into lightweight materials optimized for gas storage, photonic, and catalytic applications.

We report a general design strategy and its implementation for the synthesis and crystallization of micro- and mesoporous crystalline COFs. These materials have rigid structures, exceptional thermal stabilities (to temperatures up to 600°C), and low densities, and they exhibit permanent porosity with specific surface areas surpassing those of well-known zeolites and porous silicates. The first two members, COF-1 $[(C_3H_2BO)_6\bullet(C_9H_{12})_1]$ and COF-5 $(C_9H_4BO_2)$, can be synthesized using a simple "one-pot" procedure under mild reaction conditions that are efficient and high-yielding.

To date, attempts to prepare COFs have generally focused on synthesizing porous organic polymers with nonordered structures or densely packed linear polymers that have one-dimensional (1D) crystalline structures (*1*–*6*). An approach involving indirect multistep synthesis of open frameworks templated from molecular solids has also been pursued, but does not give crystalline or fully linked materials (*7*, *8*). Our strategy for synthesizing crystalline COFs involves using one-step condensation reactions of discrete molecules known to produce six- and five-membered rings that can be appropriated for the synthesis of their extended analogs, as shown in Fig. 1.

The synthesis of COF-1 is based on the molecular dehydration reaction (Fig. 1A), in which three boronic acid molecules converge to form a planar six-membered B_3O_3 (boroxine) ring with the elimination of three water molecules. Typically, such molecular structures of cyclotrimerized boronic acids are held in planar conformations by $C–H_{(o\text{-}C_6H_4)}\cdots O_{(BO)}$ [2.975(2) Å] hydrogen bonds (*9*). With this knowledge, we extended this reaction to 1,4-benzenediboronic acid (BDBA), in which a layered hexagonal framework was expected to form upon dehydration as shown in Fig. 1B. For COF-5, we used an analogous condensation reaction. The dehydration reaction between phenylboronic acid and 2,3,6,7,10,11-hexahydroxytriphenylene (HHTP), a trigonal building block, generates a five-membered BO_2C_2 ring (Fig. 1C). From the structural data of this discrete molecular fragment (*10*), an entirely coplanar extended sheet structure was expected to form, according to Fig. 1D.

COF-1 was synthesized by the heating of BDBA at 120°C for 72 hours under a mesitylene-dioxane solution in a sealed Pyrex tube (*11*). These conditions allowed the dehydration of BDBA to proceed slowly. The sparing solubility of BDBA in this solvent system controls the diffusion of the building blocks into solution and facilitates the nucleation of a crystalline material, whereas the use of a closed reaction system sustains the availability of H_2O for maintaining reversible conditions conducive to crystallite growth. After being heated and washed with acetone, COF-1 was isolated as a white powder in 71% yield based on BDBA. With the use of similar reaction conditions, COF-5 was synthesized in 73% yield from a 3:2 stoichiometric ratio of BDBA

[1]Materials Design and Discovery Group, Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, MI 48109–1055, USA. [2]Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287–1604, USA.

*To whom correspondence should be addressed. E-mail: oyaghi@umich.edu (O.M.Y.); apcote@umich.edu (A.P.C.)

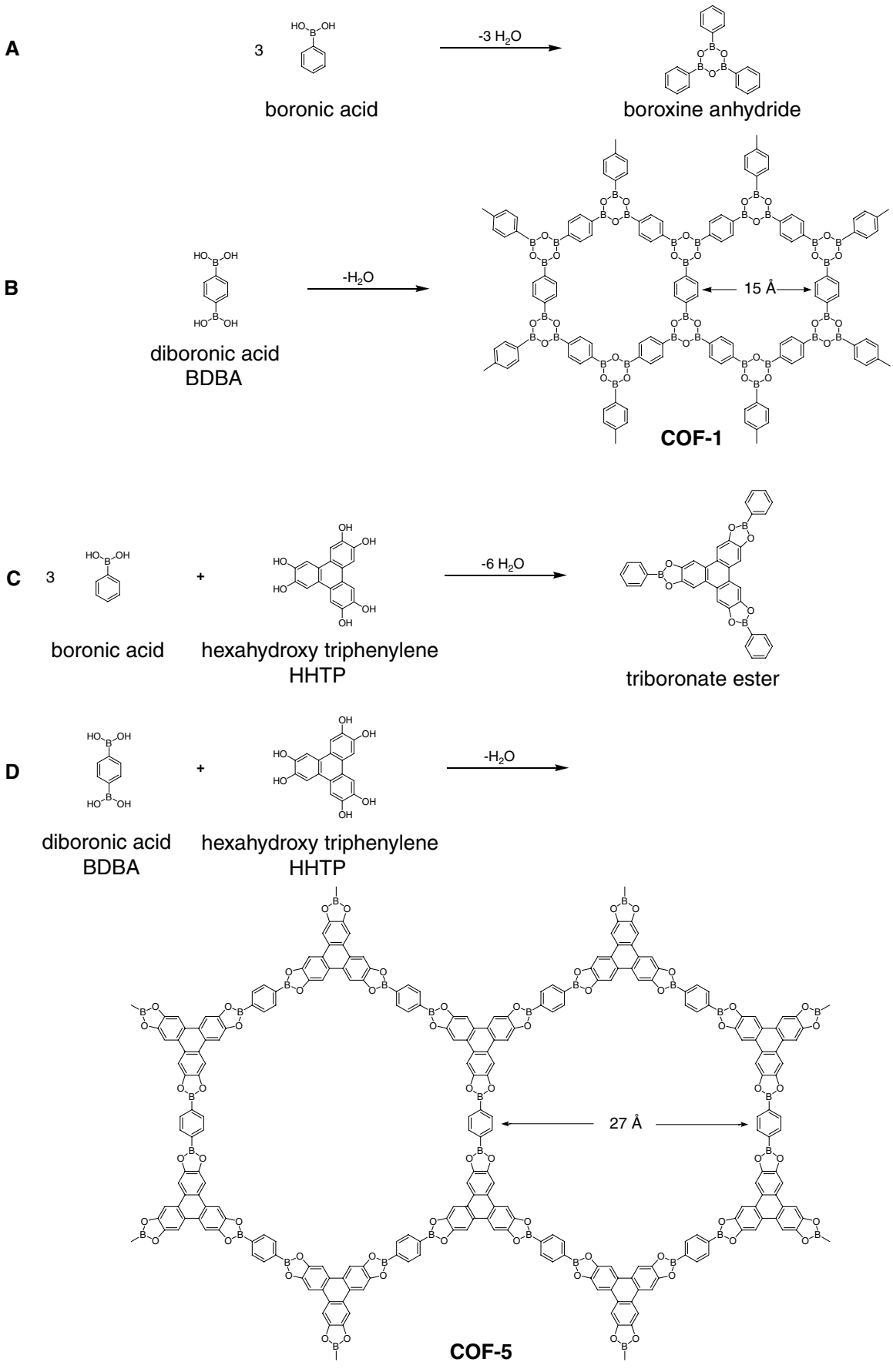


Fig. 1. (**A** to **D**) Condensation reactions of boronic acids used to produce discrete molecules and extended COFs.

to HHTP. After being washed with acetone, COF-5 was obtained as a gray-purple solid, the color of which was due to a small amount of oxidized HHTP formed during the reaction that ultimately occupied the pores of COF-5.

Powder x-ray diffraction (PXRD) analysis of both products confirmed the crystallinity of the COFs and revealed no diffraction peaks that could be attributed to starting materials or their known solvates (Fig. 2, A and E). Furthermore, the phase purity was confirmed by exhaustive scanning electron microscopy (SEM) imaging of the products from multiple reactions where only one morphologically unique crystallite could be found for each COF (Fig. 2, B and F). The Fourier transform infrared (FTIR) spectra of both materials indicated the formation of the expected boron-based ring groups in COF-1 and COF-5, displaying the bands corresponding to the respective boroxine and boronate ester rings; the hydroxyl bands of the starting materials were strongly attenuated in the COF materials (Fig. 2, C and G) (*11*). The ^{11}B solid-state nuclear magnetic resonance (NMR) spectra of the COFs, which do not match that of BDBA, were collected and found to be coincident to those obtained for the molecular model compounds shown in Fig. 1, A and C (Fig. 2, D and H). The line shapes obtained from quadripolar ^{11}B spectra are highly sensitive to the immediate chemical and geometrical bonding environment of boron (*12*). Thus, the nearly identical line shapes of COF-1 and COF-5 as compared to those of their respective boroxine and boronate ester model compounds show that the expected boron-containing rings for these materials had indeed been formed.

In order to confirm that the phases observed from PXRD measurements were indeed those targeted, we modeled the possible extended structures that could be formed from the trigonal boronic molecular units, using the *Cerius*^{2} chemical structure–modeling software suite (*13*). Simulated powder patterns were calculated from these models and compared with the experimentally observed data (Fig. 2, A and E). Given that planar 2D organic sheets were expected to form, layered crystal structures were modeled, in which two distinct stacking possibilities for the organic sheets were considered: (i) a staggered AB arrangement analogous to the packing of graphite sheets, where three-connected vertices (carbon atoms) lie over the center of the six-membered rings of neighboring graphite layers; and (ii) an eclipsed arrangement, in which atoms of adjacent sheets lie directly over each other, as in boron nitride. Like their inorganic counterparts, the staggered and eclipsed models have $P6_3/mmc$ and $P6/mmm$ symmetry, respectively, and each would exhibit distinct diffraction patterns because each space group has a distinct set of symmetry-imposed reflection conditions.

Patterns calculated from the *Cerius*^{2} models also reflect the expected peak intensities, because the x-ray scattering power, positions of framework atoms, and presence of guest molecules are also taken into account. The experimental powder patterns as compared to these calculated models are displayed in Fig. 2, A and E, where there is a close correspondence between the peak positions and intensities for the staggered graphitic model for COF-1 and the eclipsed boron nitride arrangement for COF-5, thus substantiating that these are indeed the targeted layered structures.

Indexing of the experimental x-ray patterns unambiguously gave unit cell parameters nearly equivalent to those determined from the models. To obtain the experimental values, we freely refined the unit cell parameters using full pattern decomposition and profile fitting of the diffraction patterns using a model-biased Le Bail routine [COF-1: calculated: $a = b = 15.6259$, $c = 6.7005$ Å; measured: $a = b = 15.420(1)$, $c = 6.655(4)$ Å. COF-5: calculated: $a = b = 30.0198$, $c = 3.404$ Å; measured: $a = b = 29.70(1)$, $c = 3.460(2)$] (*14*). Peak broadening, asymmetry, and zero-shift errors were accounted for in a calculated diffraction profile and refined against the observed scattering to extract the intensities (F_{obs}) for each structure. The blue difference plots, in Fig. 2, A and E, indicate that the degree of fitting is acceptable for the refined profile (including unit cell parameters).

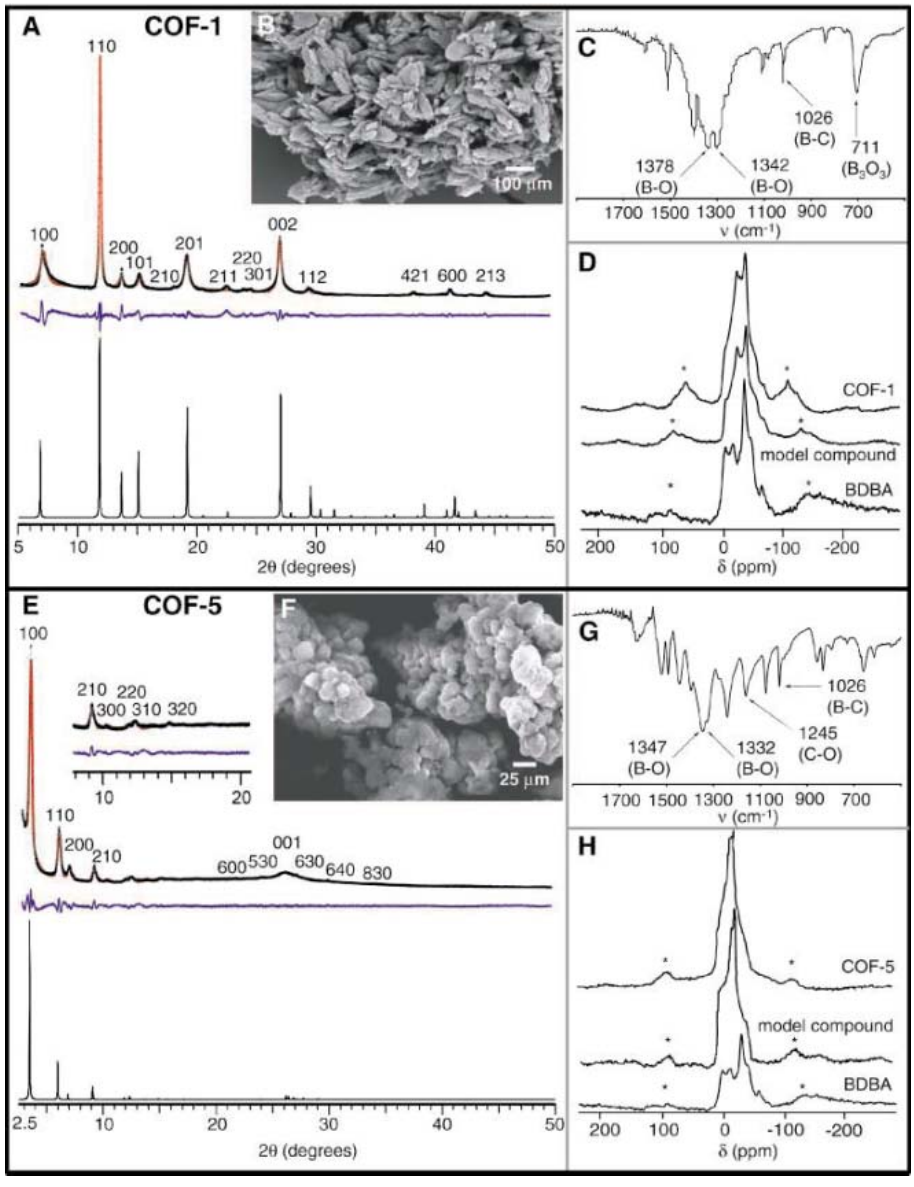


Fig. 2. Physical characterization of COF-1 (**A** to **D**) and COF-5 (**E** to **H**) (*24*). [(A) and (E)] X-ray analysis of (A) COF-1 and (E) COF-5 with the observed pattern in black, the refined profile in red, and the difference plot in blue (observed minus refined profiles). The bottom trace is the calculated PXRD pattern from *Cerius*^{2}. [(B) and (F)] SEM images of bulk COF. [(C) and (G)] FTIR spectra highlighting the characteristic boron functional group bands of (C) COF-1 and (G) COF-5. [(D) and (H)] ^{11}B magic-angle spinning NMR spectra of (top) COF, (middle) model compound, and (bottom) BDBA for (D) COF-1 and (H) COF-5. Asterisks indicate spinning side-band peaks.

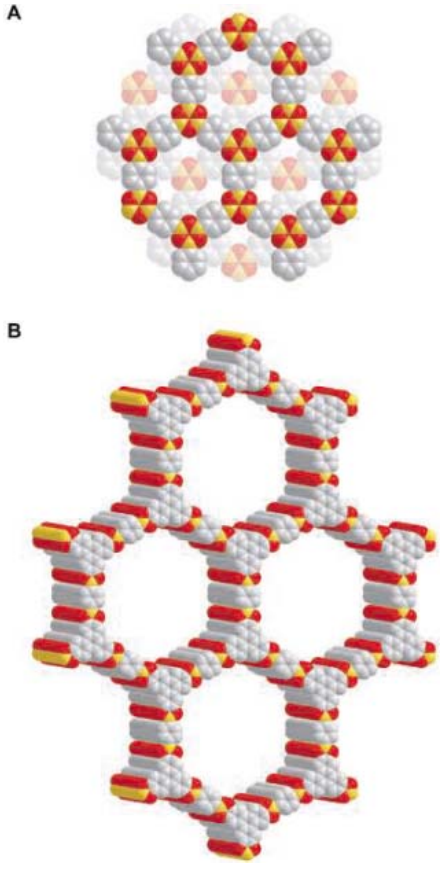


Fig. 3. Structural representations of (**A**) COF-1 and (**B**) COF-5 based on powder diffraction and modeling projected along their *c* axes (H atoms are omitted). Carbon, boron, and oxygen are represented as gray, orange, and red spheres, respectively.

The number of observed peaks for COF-1 and COF-5 were too few to permit refinement of atom positions in the models using Rietveld methods. Nevertheless, the initial intensities calculated from the *Cerius*[2] structures used in the extraction represent viable models for these data, because statistically reasonable profiles could be obtained from readily convergent refinements [COF-1: wR_p = 0.1122, R_p = 0.0871, χ^2 = 10.43 (where R_p and wR_p are profile-fitting factors); COF-5: wR_p = 0.0635, R_p = 0.0476, χ^2 = 18.46] (*15*).

The structure of COF-1 can be derived from graphite by replacing each C atom with a B_3O_3 boroxine unit and each C–C bond by a ditopic phenylene unit (Fig. 3A). As expected, the interlayer spacing of COF-1 is near that of graphite [3.328(4) versus 3.348(1) Å] (*16*). Given the presence of 15.1 Å perforations within the layers of COF-1 arising from expansion, a 1D pore exists throughout the material with an aperture of 7.0 Å defined by the van der Waals distance between phenyl and B_3O_3 rings of adjacent layers (Fig. 3A). Using ^{13}C solid-state NMR spectroscopy, we found that the pores were occupied by mesitylene guest molecules incorporated during the reaction (*11*).

To determine the thermal stability of COF-1 in the absence of guests, a thermogravimetric analysis was done on the as-synthesized materials. Mesitylene guests can be removed by heating the material to 200°C, with an accompanying mass loss of 21%; this decrease corroborates the formula determined from elemental microanalysis as $(C_3H_2BO)_6\bullet(C_9H_{12})_1$, which corresponds to one guest per cavity. There is no indication from spectroscopic analysis that degradation of the covalently bonded layers results from guest removal (*11*, *15*). COF-1 remains crystalline after evacuation of mesitylene, with some shifting of the layers being evident (fig. S21).

The structure of COF-5 can be derived from that of graphite, except that the layers stack in an eclipsed fashion as observed in boron nitride (Fig. 3B) to form a hexagonal array of 1D mesopores whose diameter is 27 Å. A notable disorder in the spacing of the layers is evident from the broad (001) peak in the powder pattern. An interlayer spacing of 3.460(2) Å (interlayer spacing = d_{001}) can be calculated and is comparable to the 3.33(1) Å spacing of boron nitride (*17*). Within examples of ordered mesoporous channel structures, periodicity at the 3 Å level has only been achieved on one occasion, in a surfactant-templated organosilicate (*18*).

We attribute the formation of an eclipsed structure over a staggered structure in this case to the presence of the HHTP unit that bestows a larger number of overriding π-π interactions to guide layer stacking rather than by B···O interactions alone. Unlike COF-1, the included guests (HHTP starting material) in COF-5 are nonvolatile. Thus, they were exchanged by soaking the as-synthesized crystals in acetone for 12 hours. The color of COF-5 changes to light gray because the highly colored oxidized form of HHTP comprises a fraction of the guests as determined from the mass spectrum of the acetone supernatant after exchange (*11*). As with COF-1, upon removal of guests, no degradation of COF-5 was evident from spectroscopic analysis, and x-ray analysis revealed that the stacking of the layers was preserved (*11*). A reliable formulation of the evacuated form of COF-5 was found by elemental microanalysis to be $C_9H_4BO_3$, corresponding to the expected formula.

The architectural stability and porosity of COF-1 and COF-5 were confirmed by measuring the N_2 gas adsorption of the guest-free material. A sample of as-synthesized COF-1 was evacuated with a dynamic 10^{-5} Torr vacuum pressure and heated to 150°C for 12 hours to remove all the guests. This sample was used for measurement of the isotherm at 77 K from 0 to 1 bar (1 bar = P_o), which showed a very sharp uptake at P/P_o from 10^{-5} to 10^{-1}, a signature feature of a microporous material (Fig. 4A). The Brunauer-Emmett-Teller (BET) model was applied to the isotherm for P/P_o between 0.04 and 0.1, which resulted in an apparent surface area of S_{BET} = 711 $m^2\ g^{-1}$; the pore volume V_p = 0.32 $cm^3\ g^{-1}$ at P/P_o = 0.90. These values surpass those of other layered materials, including graphite (10 $m^2\ g^{-1}$), clays (10 to 100 $m^2\ g^{-1}$), and pillared clays (50 to 300 $m^2\ g^{-1}$) and are in the range of the most porous zeolites and many porous carbons (*19*). At higher pressures, a slow rise in the isotherm occurs because of the existence of a small population of external mesopores between the crystallites; this feature is not uncommon for particles with platelet morphologies (*19*). The total surface area was calculated to be 711 $m^2\ g^{-1}$, with a micropore contribution of 587 $m^2\ g^{-1}$ (83%) and mesopore contribution of 124 $m^2\ g^{-1}$ (17%) from de Boer statistical thickness (*t*-plot) analysis (*19*).

We collected Ar and high-temperature CO_2 isotherms in the same pressure range, which we fit with density functional theory (DFT) models that account for the microscopic behavior of sorbed molecules (*11*, *20*). From these calculations, a very reliable pore size distribution can be calculated and is shown Fig. 4C. Corroborating the results from *t*-plot analysis, the distribution is largely populated between 6 and 12 Å and matches the micropore dimensions expected from the structure of COF-1; the range from 28 to 45 Å arises from the aforementioned interparticle mesopores. The cumulative pore volume (0.34 $cm^3\ g^{-1}$) and surface area (640 $m^2\ g^{-1}$) from DFT calculations compare favorably with the values determined above. The isotherm for COF-1 is fully reversible and reproducible: a feature of stable materials whose structures exhibit permanent porosity.

The N_2 adsorption isotherm of COF-5, measured under the same conditions as COF-1, shows a reversible type-IV isotherm characteristic of mesoporous materials (Fig. 4B). There are two notable features in this isotherm. The first is a sharp step observed for pore condensation for P/P_o from 0.11 to 0.15, caused by a narrow distribution of mesopores (*21*); this finding was supported by DFT calculations with a pore width of 27 Å dominating the distribution (Fig. 4D) (*22*). Second, the absence of hysteresis during desorption is a common feature of materials containing hexagonally aligned 1D mesopores with widths <40 Å (*21*). It is also apparent from the pore size distribution that 23% of the total surface area can be assigned to micropore uptake. Because an impurity phase has not been encountered, we speculate that the origin of the significant low-pressure uptake arises from partially slipped organic sheets that create grottos along the mesopore walls where adsorbate molecules are more strongly bound. The BET surface area of COF-5 was 1590 $m^2\ g^{-1}$, which corresponds to a mesopore volume of 0.998 $cm^3\ g^{-1}$, values that are greater than double that reported for 26 Å Mobile Crystalline Material 41 (MCM-41) (680 $m^2\ g^{-1}$

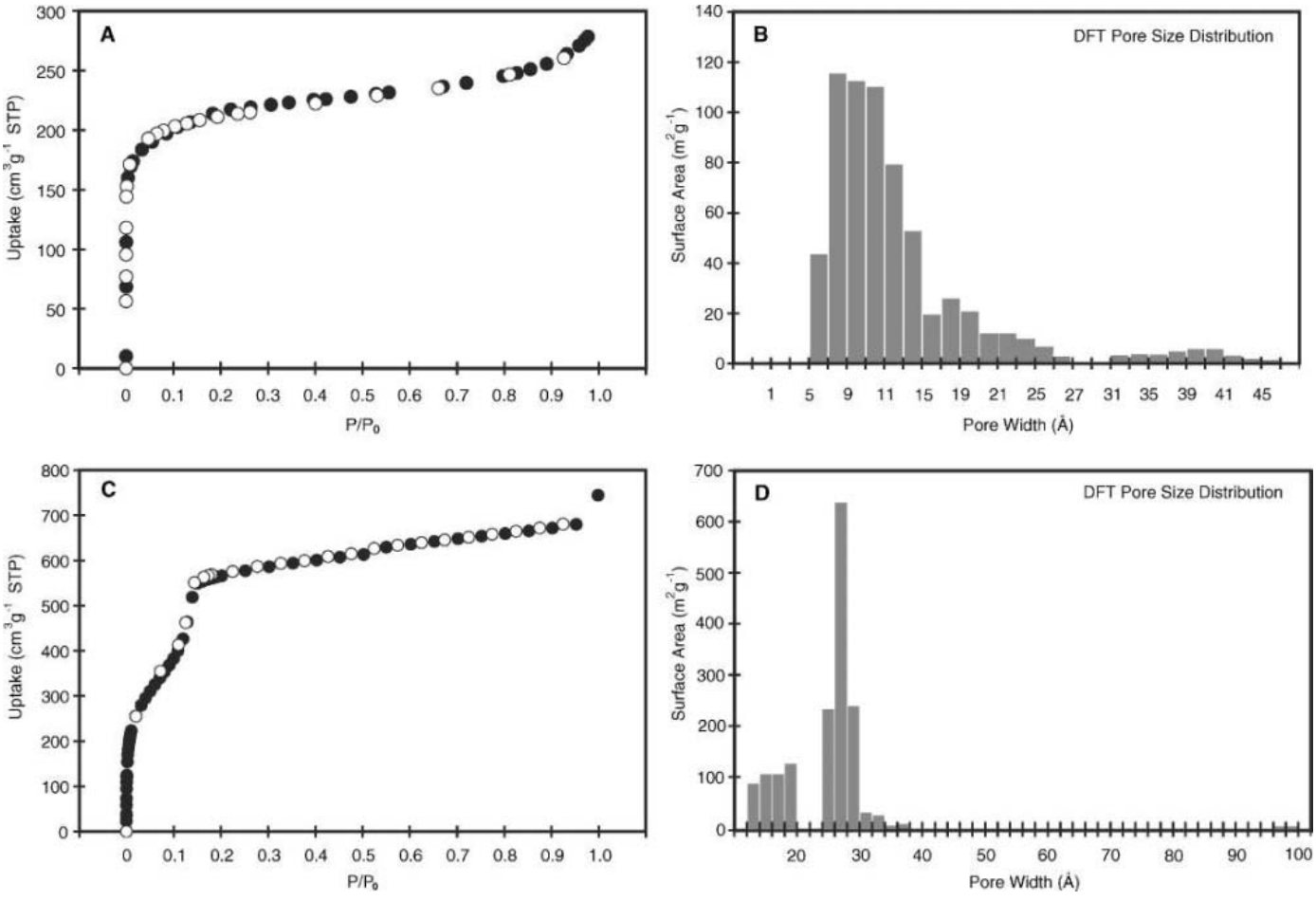


Fig. 4. Nitrogen gas adsorption isotherms for COF-1 (**A**) and COF-5 (**C**) measured at 77 K and pore size histograms for (**B**) COF-1 and (**D**) COF-5 calculated after fitting DFT models to adsorption data.

and 0.26 $cm^3\ g^{-1}$) (*23*) and exceed that of the highest reported surface area of 1300 $m^2\ g^{-1}$ for a macroporous ordered silica (*21*).

References and Notes

1. P. J. Florey, *Principles of Polymer Chemistry* (Cornell Univ. Press, Ithaca, NY, 1953).
2. D. T. Vodak *et al.*, *J. Am. Chem. Soc.* **124**, 4942 (2002).
3. H. B. Sonmez, F. Wudl, *Macromolecules* **38**, 1623 (2005).
4. S. M. Curtis *et al.*, *Supramol. Chem.* **17**, 31 (2005).
5. T. Hoang, L. W. Lauher, F. W. Fowler, *J. Am. Chem. Soc.* **124**, 10656 (2002).
6. W. Niu, C. O'Sullivan, B. M. Rambo, M. D. Smith, J. J. Lavigne, *Chem. Commun.* **2005**, 4342 (2005).
7. Z. Xu *et al.*, *Adv. Mater.* **12**, 637 (2001).
8. P. Brunet, E. Demers, T. Maris, G. D. Enright, J. D. Wuest, *Angew. Chem. Int. Ed. Engl.* **42**, 5303 (2003).
9. C. P. Brock, R. P. Minton, K. Niedenzu, *Acta Crystallogr.* **C43**, 1775 (1987).
10. V. F. Zettler, H. D. Hausen, H. D. Hess, *Acta Crystallogr.* **B30**, 1876 (1974).
11. Materials and methods are available as supporting material on *Science* Online. The synthesis of COF-1 was carried out as follows: A Pyrex tube with an outside diameter of 10 mm and an inside diameter of 8 mm was charged with BDBA (25 mg, 0.15 mmol, Aldrich) and 1 ml of a 1:1 v:v solution of mesitylene: dioxane. The tube was flash-frozen at 77 K (in a liquid N_2 bath) and evacuated to an internal pressure of 150 mTorr and flame-sealed. Upon sealing, the length of the tube was reduced to 18 cm. The reaction mixture was heated at 120°C for 72 hours, yielding a white solid at bottom of the tube, which was isolated by filtration and washed with acetone (30 ml). The yield was 17 mg, 71% for $(C_3H_2BO)_6{\bullet}(C_9H_{12})_1$. The synthesis of COF-5 was carried out as follows: Using the same reaction design as for COF-1, BDBA (12.5 mg, 0.075 mmol, Aldrich) and HHTP (16 mg, 0.050 mmol, TCI) were heated at 100°C for 72 hours to yield a free-flowing, gray-purple powder. The yield was 15 mg, 73% for $C_9H_4BO_2$ after guest removal. No evidence for the self-condensation of BDBA to form COF-1 was observed from the synthesis of COF-5. The reaction of BDBA alone at 100°C was slow, and after 168 hours of heating, produced COF-1 in only 25% yield.
12. C. Gervais, F. Babonneau, *J. Organomet. Chem.* **657**, 75 (2002).
13. *Cerius*² Modeling Environment (Molecular Simulations, San Diego, CA, 1999).
14. A. C. Larson, R. B. VonDreele, *General Structure Analysis System* (*GSAS*) (Los Alamos National Laboratory Report LAUR 86-748, Los Alamos, NM, 2004).
15. Disorder existing in these compounds arises from the stacking of the sheets, as evidenced from the line broadening in the x-ray patterns (turbostratic disorder) and is frequently observed in layered materials. The tailing to a high angle of the (100) lines points to translational faults in the *xy* basal planes of the structures, whereas the broadening of lines corresponding to layer stacking along the *c* axes [(002) for COF-1 and (001) for COF-5] indicates variances in the interlayer spacings, which is more pronounced in COF-5. The construction of the open layers in COF-1 and COF-5 has been established by spectroscopy, and analysis of their gas adsorption isotherms reveals the respective permanent micro- and mesoporosity and pore sizes near those determined from x-ray data. Despite their lack of perfect crystallinity, the investigation and use of COFs as porous materials are not precluded.
16. R. C. Tatar, S. Rabii, *Phys. Rev. B* **13**, **4126** (1982).
17. E. K. Sichel, R. E. Miller, M. S. Abrahams, C. J. Buiocchi, *Phys. Rev. B* **13**, 4607 (1976).
18. S. Inagaki, S. Guan, T. Ohsuna, O. Terasaki, *Nature* **416**, 304 (2002).
19. F. Rouquerol, J. Rouquerol, K. Sing, *Adsorption by Powders and Porous Solids* (Academic Press, London, 2002).
20. R. I. Ravikovitch, A. Vishnyakov, R. Russo, A. V. Neimark, *Langmuir* **16**, 2311 (2000).
21. M. Thommes, in *Nanoporous Materials Science and Engineering*, G. Q. Lu, X. S. Zhao, Eds. (Imperial College Press, London, 2004), chap. 11.
22. M. Kruk, M. Jaroniec, *Chem. Mater.* **12**, 222 (2000).
23. M. Kruk, M. Jaroniec, A. Sayari, *J. Phys. Chem. B* **101**, 583 (1997).
24. Atomic coordinates have been deposited in the Cambridge Crystallographic Database under deposition numbers 287138 (COF-1) and 287139 (COF-5).

25. Supported by NSF, the U.S. Department of Energy, and the Natural Sciences and Engineering Research Council of Canada (a postdoctoral fellowship to A.P.C.). We thank M. V. Wilson and L. W. Beck (University of Michigan) for their valuable assistance with NMR measurements and K. Leinenweber (Arizona State University) for his expert advice regarding x-ray analysis.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1166/DC1
Materials and Methods
Figs. S1 to S37
Tables S1 to S4

21 September 2005; accepted 21 October 2005
10.1126/science.1120411

Bright Infrared Emission from Electrically Induced Excitons in Carbon Nanotubes

Jia Chen,[1]* Vasili Perebeinos,[1] Marcus Freitag,[1] James Tsang,[1] Qiang Fu,[2] Jie Liu,[2] Phaedon Avouris[1]*

We used the high local electric fields at the junction between the suspended and supported parts of a single carbon nanotube molecule to produce unusually bright infrared emission under unipolar operation. Carriers were accelerated by band-bending at the suspension interface, and they created excitons that radiatively recombined. This excitation mechanism is ~1000 times more efficient than recombination of independently injected electrons and holes, and it results from weak electron-phonon scattering and strong electron-hole binding caused by one-dimensional confinement. The ensuing high excitation density allows us to observe emission from higher excited states not seen by photoexcitation. The excitation mechanism of these states was analyzed.

The optical properties of single-walled semiconducting carbon nanotubes (CNTs) are currently the focus of intense study (*1*–*8*). CNTs are direct band-gap materials, and their optical spectra have long been attributed to transitions between free-particle bands. Theoretical studies, on the other hand, have pointed out that there should be strong electron-hole (e^--h^+) interactions in these quasi–one-dimensional (1D) materials, resulting in strongly bound excitons (*9*–*11*). Recent experimental evidence for the formation of CNT excitons is provided by two-photon fluorescence excitation spectroscopy (*3*) and photoconductivity (*8*). CNTs offer the possibility of a unified electronic and optoelectronic technology (*12*). Therefore, it is important to understand electroluminescence (EL) from CNTs. Moreover, the study of EL provides further insights into e^--h^+ interactions in quasi-1D materials.

Here we discuss an excitation mechanism that leads to a strongly enhanced EL in the infrared (IR) from a partially suspended CNT field-effect transistor (CNTFET) operating under unipolar transport conditions. In light-emitting diodes (LEDs) or ambipolar CNTFETs, the participating carriers (both e^- and h^+) are injected from the source and drain electrodes separately (*2*, *13*). In our devices, carriers are generated locally when a single type of carrier is accelerated under high local electric field to energies sufficient to create strongly correlated e^--h^+ pairs (excitons).

This impact excitation process differs greatly in 3D and 1D systems. In bulk 3D semiconductors, the weak Coulomb interaction between e^- and h^+ creates weakly bound excitons (*14*) that mostly dissociate under high field and contribute to the electrical current (*15*). In 1D CNTs, on the other hand, exciton binding energies are predicted to be more than an order of magnitude larger (*9*–*11*), so that the 1D excitons are expected to recombine and contribute little to the current. We find that the intensity of light emission increases exponentially with the drive current in partially suspended CNTFETs [in 3D materials, light emission is usually proportional to the product of the e^- and h^+ currents (*14*)] and has a similar dependence on both the gate and drain bias, whereas the current itself increases linearly with bias.

These observations are a manifestation of the strong e^--h^+ interactions in the quasi-1D CNTs that prevent e^--h^+ pairs from dissociating and validate the exciton picture. We also find that the current-voltage (*I-V*) characteristics of the suspended CNTFETs display negative differential conductance (NDC), which, by comparison with the light emission, we find to be correlated with the onset of the generation of excitons by hot carriers (*16*). This local EL mechanism leads to a 10^2 to 10^3 times increase in efficiency over that achieved by supported ambipolar CNTFETs (*16*). From both the position of the emission spot and the dependence of the IR emission on the drain and gate bias, we conclude that the high local electrical field at the suspension interface is mainly responsible for the enhanced emission efficiency. By removing the underlying substrate and suspending the CNT, nonradiative recombination channels involving the substrate can also be reduced (*5*). The extraordinary current-carrying capability of a CNT (*12*) and its ultra-small size lead to an ultra-bright light source. Finally, the ~100 times higher exciton density (0.14 nm^{-1}) achieved in our devices, compared with that in typical photoluminescence (PL) experiments, allows us to detect emission from the second allowed exciton (E_{22}) state in CNTs. We show that E_{11} exciton-exciton annihilation is primarily responsible for the E_{22} emission. In agreement with a very recent study (*17*), where high exciton densities were achieved in PL using pulsed laser excitation, we did not see direct evidence for a Mott transition, which is expected to take place when excitons start to spatially overlap.

The CNTFETs (~25 devices) with a partially suspended CNT channel used in this study were fabricated by etching 0.4-to-15-μm-wide trenches in a 200-nm-thick SiO_2 film on Si wafers. The trenches extended through the SiO_2 film and 2 μm into the Si substrate. CNTs with diameters in the range of 2 to 3 nm were grown on the trenched substrate by chemical vapor deposition (*18*). Palladium source and drain electrodes were then patterned on CNTs with channel lengths between 4 and 80 μm. The highly doped silicon substrate was used as a back gate. Detailed experimental setup and sample fabrication are discussed in the supporting online materials (*18*).

A spatial map of the IR emission, collected with a bandpass filter centered around 1.6 μm (in the E_{11} emission band) from a single CNTFET, was superimposed on an optical image of the device structure (Fig. 1A). Unlike nonsuspended CNTFETs, whose emission from ambipolar injection can be spatially translated along the length of a CNT by changing the gate bias (*13*), the emission from the partially suspended device is localized at the SiO_2 supported/suspended CNT interfaces under a wide range of gate biases. Figure 1B shows the drain current and the emission intensity at 1.6 μm occurring at the suspension interface, as a function of the gate voltage (V_g) at a constant drain bias of $V_d = -5$ V. We observed no detectable emission when the gate bias was below a threshold gate overdrive $|V_g - V_{th}|$ of ~0.5 V, where V_{th} is the threshold voltage (–2.6 V in this sample) at which the CNTFET is electrically turned on. At $|V_g - V_{th}| > 0.5$ V, the emission intensity increases rapidly with increasing current.

There are three major differences in the IR emission from suspended CNTFETs compared with that from nonsuspended devices: (i) The dominant emission from the suspended CNT occurs under unipolar transport conditions ($V_g < -3.1$ V for h^+ and $V_g > -2.1$ V for e^-), and no appreciable emission is observed at the ambipolar region where the current shows a minimum ($V_g = -2.6$ V); (ii) The emission intensity increases exponentially with the drive current in the suspended tube (Fig. 1B, inset), whereas in a nonsuspended CNT biased under ambipolar conduction, the emission intensity is proportional to the minority carrier density (*19*); (iii) The minimum drain bias needed to observe emission is notably smaller in sus-

[1]IBM Research Division, Thomas J. Watson Research Center, Post Office Box 218, Yorktown Heights, NY 10598, USA. [2]Department of Chemistry, Duke University, Durham, NC 27708, USA.

*To whom correspondence should be addressed. E-mail: chenjia@us.ibm.com (J.C.); avouris@us.ibm.com (P.A.)

pended tubes (V_d ~ 2.5 V) than that in nonsuspended tubes (V_d ~ 15 V) (*13*). In turn, a CNTFET operated under ambipolar conditions needs to be driven to higher drain/gate fields and currents to generate the same amount of light as the suspended CNT, indicating that EL via ambipolar conduction is a much less efficient process. Indeed, the emission efficiency defined as photons per electron measured on the suspended samples (*16*) is about 10^{-3} to 10^{-4}, that is, the partially suspended samples exhibit an increase of 2 to 3 orders of magnitude from that of supported CNTs operated under ambipolar conduction conditions (*16*). Although the efficiency (photons/electron) of the suspended CNTs is numerically comparable to that of CNTs wrapped in micelles in PL measurements (emitted photons/adsorbed photon) (*1*), the small optical cross section of CNTs (10^{-11} to 10^{-12} cm^2 per CNT) (*20*) reduces the effectiveness of exciton generation by photoexcitation. For a typical injection current of 3 μA, ~20 electrons per ps are injected in a steady state in a CNT, generating ~0.02 photons per ps by EL; correspondingly, ~2×10^{13} cm^{-2} ps^{-1} per CNT input photons are required by photoexcitation. The small emitting area coupled with the high current-density–carrying capability in a CNT makes it possible to produce an ultra-bright nano–light emitter. For example, a 3-μA current in a partially suspended CNTFET generates about 10^7 photons nm^{-2} s^{-1}.

How is the light generated in the supported/suspended CNT interface? Figure 1C shows a schematic band diagram of a partially suspended CNT with a gate biased to produce unipolar conduction. At the junction between the SiO_2-supported part of the CNT and the part suspended over the trench, the decrease in dielectric constant (*21*) leads to a reduced capacitive coupling to the Si back gate. This process gives rise to a bending of the CNT bands, which, together with the source-drain field, generates a high local electric field $\mathcal{E}$ and produces hot carriers. Thus, electrons injected into the conduction band are accelerated toward the source by the high field at the interface. If an electron can be accelerated to a sufficiently high energy and does not lose that energy by optical phonon scattering, it can create an exciton that can decay radiatively. The electron that lost energy to the generated exciton could, in turn, pick up energy from the field and continue this process. For an impact excitation process to occur, both energy and momentum need to be conserved. The threshold energy should be at least equal to the lowest exciton energy, and the need to conserve momentum typically increases the threshold energy by a factor of ~1.5 (*22*). Exciton-band–mixing effects (*10*, *11*) and interactions with the substrate can, however, relax the momentum conservation law, and the threshold energy can be as low as the exciton energy.

A rough estimate of the threshold electrical field $\mathcal{E}_{th}$ needed for impact excitation is given by $1.5E_g/\lambda_{ph}$, where E_g is the transition energy [optical band gap ~ 0.56 eV for a 1.9-nm-diameter CNT (*23*)] and $\lambda_{ph} \approx 10$ to 20 nm is the optical phonon scattering length reported in CNTs (*24*, *25*). Compared with 3D materials (*22*), the longer optical phonon scattering length implies a lower threshold field for the onset of impact excitation in 1D CNTs, whereas the larger exciton binding energy in 1D CNTs prevents e^--h^+ pairs from dissociating. These two factors make emission from impact excitation in 1D a more probable process than that in 3D materials. Indeed, $\mathcal{E}_{th}$ for the 1D CNT is estimated to be ~$1.5E_g/\lambda_{ph}$ = 0.3 to 0.6 MV/cm, i.e., it is ~7 times smaller than that in bulk materials with the same excitation energy (*22*).

The emission intensity generated by the high interface field $\mathcal{E}_{int}$ should be proportional to the impact excitation rate (*22*), $\exp(-\mathcal{E}_{th}/\mathcal{E}_{int})$. The probability for an electron to travel without scattering a distance L is given by $\exp(-L/\lambda_{ph})$. Therefore, the probability to accelerate an electron to energy E_{th} is $\exp(-E_{th}/e\mathcal{E}_{int}\lambda_{ph})$, where e is the electron charge and where field $\mathcal{E}_{int}$ has contributions from both the band-bending and the source-drain fields, $\mathcal{E}_{band}$ and $\mathcal{E}_{sd}$, respectively. $\mathcal{E}_{band}$ can be estimated as $\Delta E_c/\lambda_{scr} \sim [\alpha_{sub}(V_g - V_{th}) - \alpha_{sus}(V_g - V_{th})]/\lambda_{scr}$ (*26*), where ΔE_c is the band bending, α_{sub} is the coupling between the gate and the substrate-supported CNT, α_{sus} is the coupling between the gate and the suspended CNT, and λ_{scr} is the screening length (*27*). $\mathcal{E}_{sd}$ is the contribution from the source-drain field that accelerates the carriers, and can be written as $\sim\gamma V_d/\lambda_{scr}$, where γ is the fraction of the source-drain field that contributes to the SiO_2 supported/suspended interface junction. The IR emission intensity of the radiatively recombining e^--h^+ pairs is proportional to the carrier impact excitation rate

$$\exp(-\mathcal{E}_{th}/\{[(\alpha_{sub} - \alpha_{sus}) \times (V_g - V_{th}) + \gamma V_d]/\lambda_{scr}\}) \quad (1)$$

We first examine the dependence of emission intensity on the gate bias. Figure 2A shows the drain current of another suspended device and the corresponding IR emission intensity at 1.6 μm (within the E_{11} emission band) versus gate voltage V_g at drain biases of V_d = 6 and 7 V. IR emission occurs at a gate voltage overdrive of ~2 V. The IR emission is localized at the supported/suspended interface (Fig. 2A, inset). The emission intensity indeed shows the dependence on the gate field predicted by Eq. 1 and can be fit for both drain biases, as shown by the solid curves in Fig. 2A. From the fitting, a γ of 0.05 is obtained at both drain biases. A band-bending of ~0.4 eV at the peak of light emission can be estimated from electrostatics (*27*). $\mathcal{E}_{int}$ in our device is estimated to be 0.01 to 0.2 MV/cm for the fields at the onset and the peak of light emission.

We now turn to the dependence of light emission on the source-drain field. Figure 2B shows the drain current I_d and light emission at 1.6 μm versus drain bias V_d at constant gate biases of V_g = 0 and 1 V of the same device. Unlike CNTs on solid substrates (*13*), the following is true for suspended devices: (i) Approximately 6 times lower drain bias is sufficient under unipolar conduction to generate light emission, benefiting from the high local field generated by the band-bending at the suspended/supported CNT interface. (ii) The suspended devices show NDC in their $I_d - V_d$ characteristics. The appearance of NDC correlates with the observation of IR emission (Fig. 2B). At its onset, impact excitation is expected to generate nonemitting triplet excitons followed, at higher energy, by light-emitting singlet excitons (*28*). The observed NDC could be

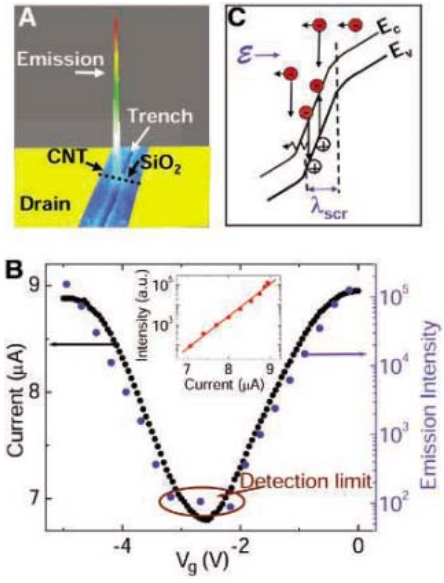


Fig. 1. (**A**) E_{11} infrared emission overlaid with an optical image of a partially suspended CNTFET with 200 nm SiO_2, Pd source/drain contacts and a Si back gate. The length of the channel is 26 μm, the width of the trench is 5 μm, and the CNT diameter is ~2 nm. (**B**) Drain current (solid squares) and emission intensity (solid circles) versus gate bias at 1.6 μm of a partially suspended CNTFET at V_d = –5 V. One unit of emission intensity corresponds to about 240 photons per second per 4π solid angle in all the following figures. The inset shows the emission intensity increases exponentially with drive current. The solid line is meant as a guide to the eyes. (**C**) Schematics of impact excitation process. An incoming hot electron is accelerated by the band-bending at the suspended/supported interface to energies larger than the band gap and generates an exciton that decays radiatively. Subsequently, the "cooled" electron picks up more energy from the electrical field and continues this process.

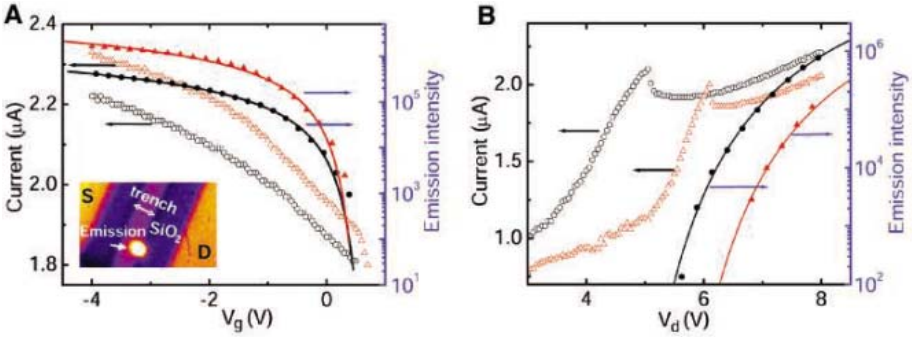


Fig. 2. (**A**) Drain current (open circles and triangles) and emission intensity versus V_g at 1.6 μm of another partially suspended CNTFET at V_d = 6 V (open and solid circles) and V_d = 7 V (open and solid triangles). The solid curves are fits to the intensity versus gate bias according to Eq. 1. The inset shows E_{11} IR emission overlaid with an optical image of the partially suspended CNTFET. The length of the CNTFET channel is 28 μm, the width of the trench is 10 μm, and the CNT diameter is ~2 nm. (**B**) Drain current (open circles and triangles) and emission intensity (solid circles and triangles) versus V_d at 1.6 μm of the same device at V_g = 0 V(open and solid circles) and V_g = 1 V (open and solid triangles). The solid curves are fits to the intensity with drain bias according to Eq. 1. (**C**) E_{11} exciton generation rate r_{11} as a function of electric field for the three zig-zag tubes with diameters d = 1.5 (red circles), 2.0 (blue squares), and 2.5 nm (green triangles). We define r_{11} as the generation rate of electron-hole pairs with energy below the second band, which is averaged over the nonequilibrium distribution (from the Boltzmann equation solution) and normalized per single carrier (*18*). The solid curves are the best fits to $r_{11} \propto \exp(-\mathcal{E}_{th}/\mathcal{E})$ with the following threshold fields: $\mathcal{E}_{th}$ = 0.41, 0.22, and 0.14 MV/cm, respectively. The inset shows the diameter dependence of the threshold field and a power-law fit $\mathcal{E}_{th} = 0.9/d^{-2}$ MV/cm (d in nm) for the zig-zag tubes with mod (n – m,3) = 1. mod (n – m,3) gives a remainder in the division of n – m by 3, where n and m are the chiral indices of a CNT. Zig-zag tubes with mod (n – m,3) = –1 have 15% smaller $\mathcal{E}_{th}$.

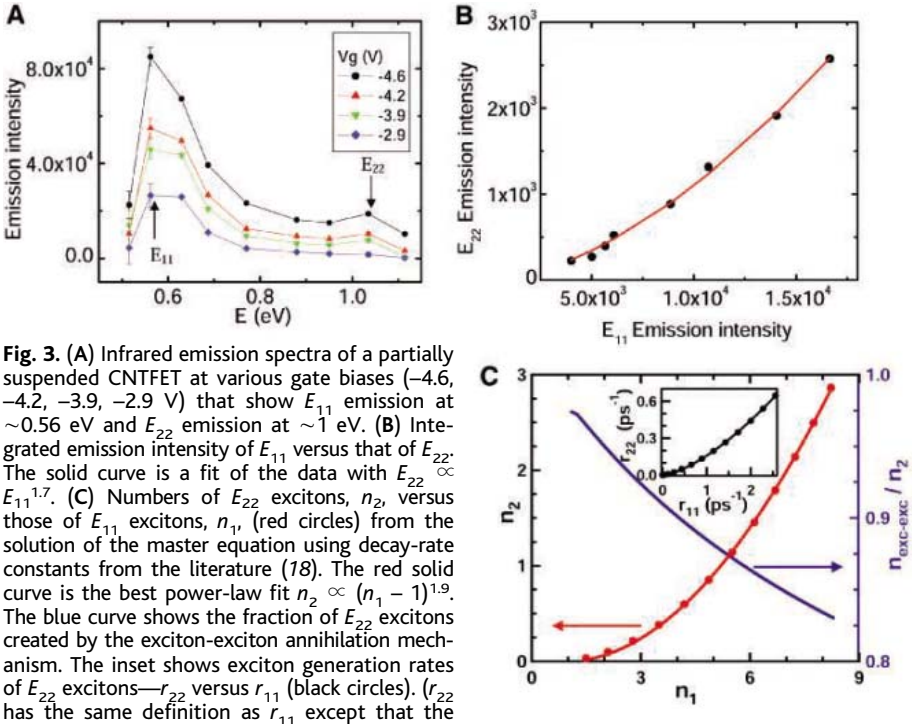


Fig. 3. (**A**) Infrared emission spectra of a partially suspended CNTFET at various gate biases (–4.6, –4.2, –3.9, –2.9 V) that show E_{11} emission at ~0.56 eV and E_{22} emission at ~1 eV. (**B**) Integrated emission intensity of E_{11} versus that of E_{22}. The solid curve is a fit of the data with $E_{22} \propto E_{11}^{1.7}$. (**C**) Numbers of E_{22} excitons, n_2, versus those of E_{11} excitons, n_1, (red circles) from the solution of the master equation using decay-rate constants from the literature (*18*). The red solid curve is the best power-law fit $n_2 \propto (n_1 - 1)^{1.9}$. The blue curve shows the fraction of E_{22} excitons created by the exciton-exciton annihilation mechanism. The inset shows exciton generation rates of E_{22} excitons—r_{22} versus r_{11} (black circles). (r_{22} has the same definition as r_{11} except that the electron-hole pair energy is above the second band.) The solid black curve is the best power-law fit $r_{22} = 0.157 r_{11}^{1.5}$ (r is in ps^{-1}) used in simulations of n_2 and n_1.

attributed to the momentum loss from impact excitation of excitons. In a gate scan where gate overdrive $|V_g - V_{th}|$ is a variable (Fig. 1C and Fig. 2A), the momentum loss from the charged carriers that have undergone impact excitation can be compensated by the increase in the carrier density caused by the increasing gate overdrive. Therefore, no further reduction of current is observed. The light-emission intensity shows a dependence on the drain field that is also in accord with Eq. 1 and can be fit for both gate biases as shown by the solid curves in Fig. 2B. By biasing the device at different gate overdrives with $|V_g - V_{th}| \approx 2$ and 1 V for V_g = 0 and 1 V, respectively, we find that indeed a higher drain field is necessary ($\Delta V_d = +1$ V) to compensate a lower gate overdrive ($\Delta|V_g - V_{th}| \approx -1$ V) in order for impact excitation to take place.

To understand the voltage dependences of the light-emission intensity, we calculated the exciton generation rate as a function of the electric field by solving the Boltzmann equation in the presence of phonon and impact excitation scattering for the charge-carrier distribution function (*18*). The results of the calculations are shown in Fig. 2C for three CNTs with different diameters along with the best fit to $I \propto \exp(-\mathcal{E}_{th}/\mathcal{E})$ predicted by the simple model. In agreement with experiment, impact excitation does not take place for fields below 0.02 to 0.03 MV/cm. The diameter dependence of the threshold field can be well approximated by a power law $\mathcal{E}_{th} \propto E_g/\lambda_{ph} \propto d^{-2}$ (Fig. 2C, inset), as a result of both the inversely proportional dependence between E_g and the CNT diameter d and the proportional dependence between λ_{ph} and d. We calculate λ_{ph} here as the ratio of the impact excitation threshold energy to the threshold field and find it in the range of 20 to 40 nm (for d =1.5 to 2.5 nm CNTs), in accord with previous studies (*24*, *25*, *29*).

The high local density of excitons produced at the suspended/supported CNT interface allows us to directly observe exciton-exciton interactions. Studies of the dynamics of excitation decay in photoexcited CNTs in micelles have shown a nonexponential, laser power–dependent decay, whose short time component was attributed to Auger quenching involving free carriers (*3*, *30*) or exciton-annihilation processes (*31*, *32*). In addition to the exciton-exciton interaction, direct generation of E_{22} excitons by hot carriers is possible in EL. By examining the evolution of the EL spectrum as a function of the gate voltage and from the results of our simulations, we conclude that E_{11} exciton-exciton annihilation is primarily responsible for the production of E_{22} excitons. Figure 3A shows the spectra of a partially suspended, 1.9-nm-diameter tube at various gate biases and a fixed V_d of –5 V. The strong emission peak at 0.56 eV corresponds very well with the lowest optically active transition of the CNT, E_{11}, observed in PL experiments (*23*).

In the EL experiments, however, a weaker peak near 1 eV is also observed. The energy of the transition lies at the expected position of the second optically allowed transition, E_{22}, of the CNT [with $E_{11}/E_{22} \sim 1.8$, also consistent with that of PL studies (*23*)]. The nature of the excitation mechanism of this state is revealed by examining the light emission intensity of E_{22} as a function of the intensity of E_{11} and the results of our simulations (*18*). Figure 3B shows that $I(E_{22}) \propto I(E_{11})^{1.7}$. This nonlinear dependence alone cannot distinguish between a direct exciton-generation mechanism and a "bimolecular" exciton-exciton annihilation [$E_{11} + E_{11} \rightarrow E_{22}$ (+ phonons)] process. Indeed, the direct impact excitation rate of E_{22} also has a power-law dependence on E_{11} generation (Fig. 3C, inset). Further carrier-kinetics calculations using the master equation (*18*) indicate that the formation of E_{22} excitons is primarily caused by the annihilation of two E_{11} excitons. There is a negligible E_{22} exciton density until the exciton-exciton annihilation process becomes possible (Fig. 3C), which is consistent with the experimental data in Fig. 3A. The fraction of E_{22} excitons generated by the exciton-exciton annihilation mechanism gradually decreases with increasing field, from 100% to slightly above 80% for the typical fields achieved in our devices. From the master equation (*18*), we estimate that there are about eight E_{11} excitons at the intrananotube junction at a field of 0.15 MV/cm. The total exciton density (0.14 nm^{-1}) is therefore about 100 times larger than what has been reported in typical PL experiments (*32*), making it possible to observe the "bimolecular" process. Such annihilation processes are widely observed in molecular crystals, polymers, and j-molecular aggregates (*33*), and apparently play a key role in the photo- and electroluminescence of CNTs.

References and Notes

1. S. M. Bachilo *et al.*, *Science* **298**, 2361 (2002).
2. J. A. Misewich *et al.*, *Science* **300**, 783 (2003).
3. F. Wang, G. Dukovic, L. E. Brus, T. F. Heinz, *Science* **308**, 838 (2005).
4. A. Hagen, G. Moos, V. Talalaev, T. Hertel, *Appl. Phys. A* **78**, 1137 (2004).
5. J. Lefebvre, Y. Homma, P. Finnie, *Phys. Rev. Lett.* **90**, 217401 (2003).
6. H. Htoon, M. J. O'Connell, S. K. Doorn, V. I. Klimov, *Phys. Rev. Lett.* **94**, 127403 (2005).
7. S. G. Chou *et al.*, *Phys. Rev. Lett.* **94**, 127402 (2005).
8. X. Qiu *et al.*, *Nano Lett.* **5**, 749 (2005).
9. T. Ando, *J. Phys. Soc. Jpn.* **66**, 1066 (1997).
10. C. D. Spataru, S. Ismail-Beigi, L. X. Benedict, S. G. Louie, *Phys. Rev. Lett.* **92**, 077402 (2004).
11. V. Perebeinos, J. Tersoff, P. Avouris, *Phys. Rev. Lett.* **92**, 257402 (2004).
12. P. Avouris, *Mater. Res. Soc. Bull.* **29**, 403 (2004).
13. M. Freitag *et al.*, *Phys. Rev. Lett.* **93**, 076803 (2004).
14. P. Y. Yu, M. Cardona, *Fundamentals of Semiconductors* (Springer, New York, 1996).
15. C. Tedesco *et al.*, *IEEE Trans Elec. Dev* **40**, 1211 (1993).
16. M. Freitag *et al.*, *Nano Lett.* **4**, 1063 (2004).
17. G. N. Ostojic *et al.*, *Phys. Rev. Lett.* **94**, 97401 (2005).
18. Details of sample fabrication, experimental methods, and simulations are available as supporting material on *Science* Online.
19. S. Heinze, J. Tersoff, P. Avouris, in *Introducing Molecular Electronics*, G. Cuniberti, G. Fagas, K. Richter, Eds. (Springer, New York, 2005), in press.
20. M. F. Islam, D. E. Milkie, C. L. Kane, A. G. Yodh, J. M. Kikkawa, *Phys. Rev. Lett.* **93**, 37404 (2004).
21. Trapped charge in silicon dioxide film could also contribute to CNT band-bending.
22. Y. Okuto, C. R. Crowell, *Phys. Rev. B* **6**, 3076 (1972).
23. B. Weisman, S. Bachilo, *Nano Lett.* **3**, 1235 (2003).
24. A. Javey *et al.*, *Phys. Rev. Lett.* **92**, 106804 (2004).
25. J. Y. Park *et al.*, *Nano Lett.* **4**, 517 (2004).
26. The band offset at the suspension/substrate interface, ΔE_c, can be estimated from its coupling to the gate as $[\alpha_{sub}(V_g - V_{th}) - \alpha_{sus}(V_g - V_{th})]$, where α is the coupling between the gate bias and the band energy and can be estimated as $C_{el}/(C_{el} + C_q)$. Here C_{el} is the electrostatic Si back gate–coupling capacitance, and $C_{el} = 2\pi\varepsilon\varepsilon_0 / \ln(4t/d)$, where ε is the dielectric constant, ε_0 is the vacuum permittivity, d is the CNT diameter, and t is the thickness of the gate dielectric. For a CNT with d = 1.9 nm on a 200-nm-thick SiO_2 (ε = 3.9), $C_{el,substrate}/L \sim 0.36$ pF/cm; when the CNT is suspended 2 μm away from the gate, $C_{el,suspended}/L \sim 0.07$ pF/cm. C_q is the quantum capacitance that is in series with the electrostatic capacitance (*34*), proportional to the average density of states [DOS, $D(E)$] of the nanotube, $C_q = dQ/dE \approx e^2D(E) \approx 4e^2/\hbar\pi v_F = 4$ pF/cm, where Q is the charge on a CNT, $\hbar$ is the reduced Planck's constant, and v_F is the Fermi velocity of graphene. This DOS is much larger than the electrostatic capacitance. In our device, $\alpha_{sub} \sim 0.09$ and $\alpha_{sus} \sim 0.02$.
27. λ_{scr} can be roughly estimated as $(\varepsilon_{cnt}d_{cnt}d_{ox}/\varepsilon_{ox})^{0.5}$ for the transistor geometry (*35*), where ox indicates oxide, and its value is on the order of 20 to 40 d_{cnt} in our devices. ε_{cnt} can be estimated from (*36*).
28. V. Perebeinos, J. Tersoff, P. Avouris, *Nano Lett.*, in press.
29. V. Perebeinos, J. Tersoff, P. Avouris, *Phys. Rev. Lett.* **94**, 086802 (2005).
30. F. Wang, G. Dukovic, L. E. Brus, T. F. Heinz, *Phys. Rev. Lett.* **92**, 177401 (2004).
31. J. Kono *et al.*, *Appl. Phys. A* **78**, 1093 (2004).
32. Y. Ma, L. Valkunas, S. L. Dexheimer, S. M. Bachilo, G. R. Fleming, *Phys. Rev. Lett.* **94**, 157402 (2005).
33. H. van Amerongen, L. Valkunas, R. van Grondelle, *Photosynthetic Excitons* (World Scientific, Singapore, 2000).
34. A. Rahman, J. Guo, S. Datta, M. S. Lundstrom, *IEEE Trans. Electron Dev.* **50**, 1853 (2003).
35. R. H. Yan, A. Ourmazd, K. F. Lee, *IEEE Trans Electron Dev.* **39**, 1704 (1992).
36. L. X. Benedict, X. G. Louie, M. L. Cohen, *Phys. Rev. B* **52**, 8541 (1995).

37. We thank J. Rosner and Central Scientific Services for help with the optical mask design, and B. Ek for expert technical assistance.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1171/DC1
Materials and Methods
References

22 August 2005; accepted 24 October 2005
10.1126/science.1119177

Retention of Xenon in Quartz and Earth's Missing Xenon

Chrystèle Sanloup,[1]* Burkhard C. Schmidt,[2]† Eva Maria Chamorro Perez,[3] Albert Jambon,[1] Eugene Gregoryanz,[4] Mohamed Mezouar[5]

The reactivity of xenon with terrestrial oxides was investigated by in situ synchrotron x-ray diffraction. At high temperature (T > 500 kelvin), some silicon was reduced, and the pressure stability of quartz was expanded, attesting to the substitution of some xenon for silicon. When the quartz was quenched, xenon diffused out and only a few weight percent remained trapped in samples. These results show that xenon can be covalently bonded to oxygen in quartz in the lower continental crust, providing an answer to the missing xenon problem; synthesis paths of rare gas compounds are also opened.

The atmospheres of Earth and Mars are depleted in Xe by a factor of 20 relative to other rare gases (i.e., Ne, Ar, and Kr) (*1*). More than 99% of Xe was degassed from the mantle (*2*), and the core seems an improbable Xe reservoir (*3*), suggesting that the "missing" Xe must be trapped elsewhere. Ices (*4*), clathrates (*5*), and sediments (*6*) were first tested as potential Xe reservoirs, without success. Early escape from the atmosphere was suggested to explain the light-isotopic abundance pattern (*7*) of atmospheric Xe, but hydrodynamic escape models require that most of the primordial Xe be retained in the interiors of the planets (*8*). This Xe retention could be explained if the normally inert Xe became increasingly soluble or even formed compounds under the conditions found within planetary interiors, resulting in the depletion of Xe in the atmosphere. Experimentally, no reaction has been observed between Xe and Fe at pressures up to 70 GPa (*3*), and if Xe were denser than mantle materials (*9*), its extremely low concentration in the mantle leads us to question how gravitationally unstable particles are formed. The incompatible character of rare gases may vary with pressure (P) and temperature (T), and it

[1]Université Pierre et Marie Curie and Institut de Physique du Globe de Paris, 75252 Paris, France. [2]Bayerisches GeoInstitut, 95440 Bayreuth, Germany. [3]Laboratoire de Sciences de la Terre, Ecole Normale Supérieure de Lyon, 69364 Lyon, France. [4]School of Physics and Centre for Science at Extreme Conditions, University of Edinburgh, EH9 3JZ, UK. [5]European Synchrotron Radiation Facility, 38043 Grenoble, France.

*To whom correspondence should be addressed. E-mail: sanloup@ccr.jussieu.fr
†Present address: Geowissenschaftliches Zentrum, Goettingen University, 37077 Goettingen, Germany.

has been suggested that Ar is compatible in silica melts (*10*). However, for most complex compositions, rare gases are thought to remain incompatible (*10*–*12*). Here we show that Xe can substitute for Si in quartz at high *P* and *T*.

Xe has the highest polarizability and consequent reactivity among rare gases. Xe was first found to bond with F, then with O (XeO_3, XeO_4, perxenates); both XeO_3 and XeO_4 are thermodynamically unstable compounds that decompose explosively. The chemistry of Xe now extends to C, N, S, and halogens (*13*). However, Xe compounds have not yet been synthesized from major terrestrial materials, with the exception of the encapsulation of Xe in caged materials such as clathrate hydrates, some of which are stable up to a few GPa (*14*), or zeolites.

We conducted in situ x-ray diffraction studies at high *P* and *T* (0.5 to 6 GPa and 300 to 2300 K) using a Paris-Edinburgh cell (at the European Synchrotron Radiation Facility, beamline ID30) and ex situ multianvil runs at higher pressures (10 GPa and 2300 K). Xe was gas loaded on the top of SiO_2 powder in a sealed and inert capsule made of Pt (*15*), which is a heavily x-ray–absorbant material. To reduce absorption of the capsule material, the monochromatic x-ray beam was tuned to high energy E = 78.1 keV, given that the absorption coefficient μ, increases with wavelength for a given element.

Upon compression at ambient temperature, solid Xe was identified in the x-ray diffraction spectra (Fig. 1). For quartz, when *T* increased, the solid Xe signal was lost and no liquid Xe signal was ever observed. Liquid Xe is characterized by large interatomic distances [~4 Å (*16*)] and should contribute to the signal at low angles. However, we loaded a few capsules with magnesiowüstite + Xe. In these cases, a liquid Xe signal was observed at the same conditions between 2.5° and 3.5° (Fig. 1). The lowest temperature at which the solid Xe signal was lost was 500 K at 0.7 GPa. Above 1100 K, a liquid was systematically observed for SiO_2 + Xe runs, between 3.5° and 6° below the solid Pt first diffraction peak, i.e., where molten Pt (melting temperature T_m = 2041 K at room pressure) would be expected.

Whatever phase of silica was loaded (cristobalite or glass), quartz was the observed stable phase throughout most of the investigated *P* and *T* range (Fig. 2). On the high-temperature side of the α-quartz stability field, β- and α-quartz could not be distinguished on the basis of diffraction patterns, but the observed negative thermal expansivity above 1000 K implies the presence of β-quartz. On the high-pressure side, quartz was observed as the main silica phase all the way up to 5.5 GPa. Above 4 GPa, additional coesite peaks were observed, but with a low intensity relative to quartz peaks (Fig. 1), attesting to the limited transformation of quartz into coesite and the extended stability field of quartz in the presence of Xe. However, the quartz-coesite transition should have been fully completed within 80 min, and half completed within 10 min [according to kinetic studies conducted at 3 GPa and 1173 K (*17*)], and at an even faster rate at higher temperatures. Furthermore, the observed volume of either α-quartz or β-quartz was ~2% larger than predicted by their equations of state. This marked extension of the quartz stability field, along with its increased cell volume, is a good indicator that there is an interaction between quartz and Xe.

Scanning electron microscope analyses of quenched samples revealed the presence of Pt + Si zones on the border of the capsule or dispersed throughout the silica matrix (Fig. 3). The crystallographic structure of Pt_3Si is

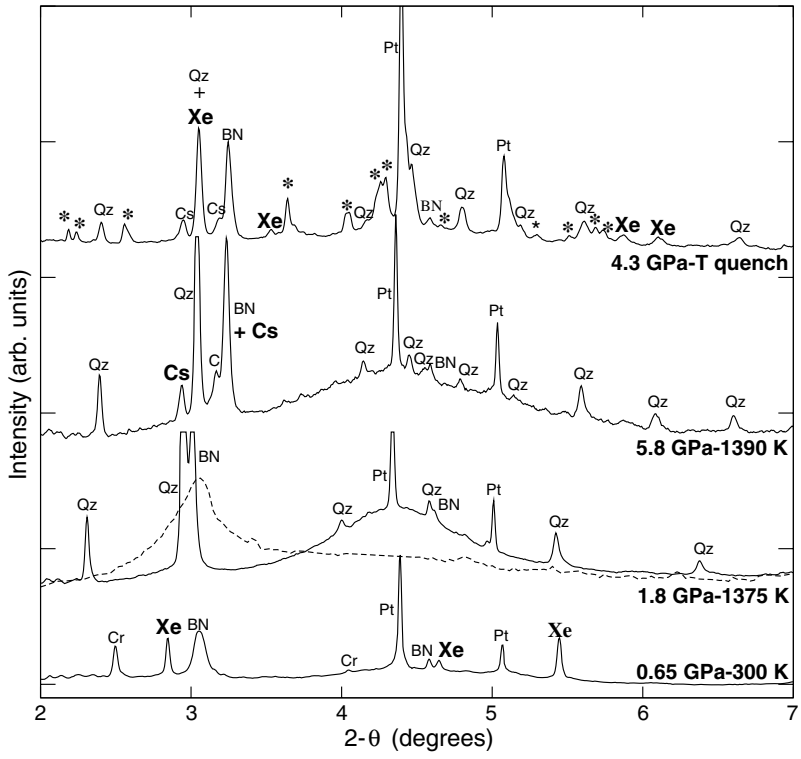


Fig. 1. X-ray diffraction spectra. Asterisks denote Pt_3Si peaks; Cr, cristobalite; Qz, quartz; Cs, coesite; C, graphite (heater); BN, boron nitride; arb., arbitrary. The dashed curve indicates the Xe liquid signal as observed in a magnesiowüstite + Xe experiment, added for comparison with the diffuse signal observed above 1100 K.

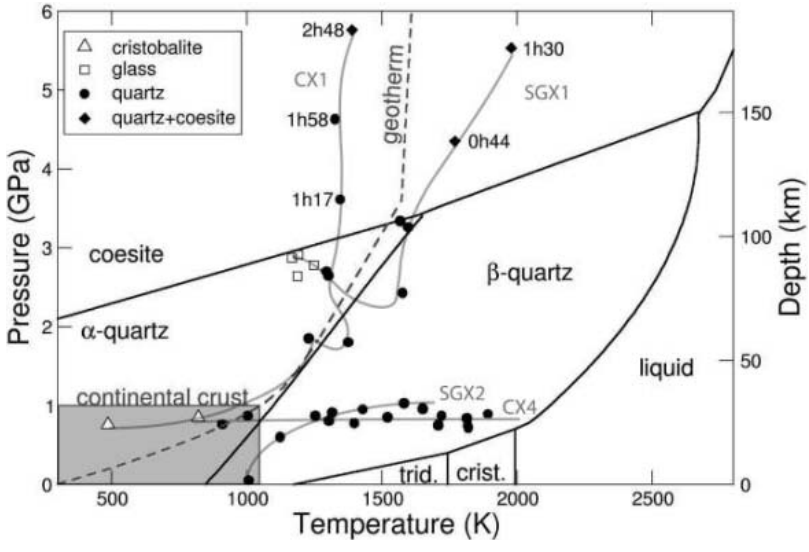


Fig. 2. Phase diagram of silica for *P* and *T* conditions of in situ x-ray diffraction experiments. Gray lines represent the experimental path for each sample. CX1 and CX4, cristobalite and Xe; SGX1 and SGX2, glass and Xe. The time values indicate how long the samples were above the normal quartz-coesite transition. crist., cristobalite; trid., tridymite.

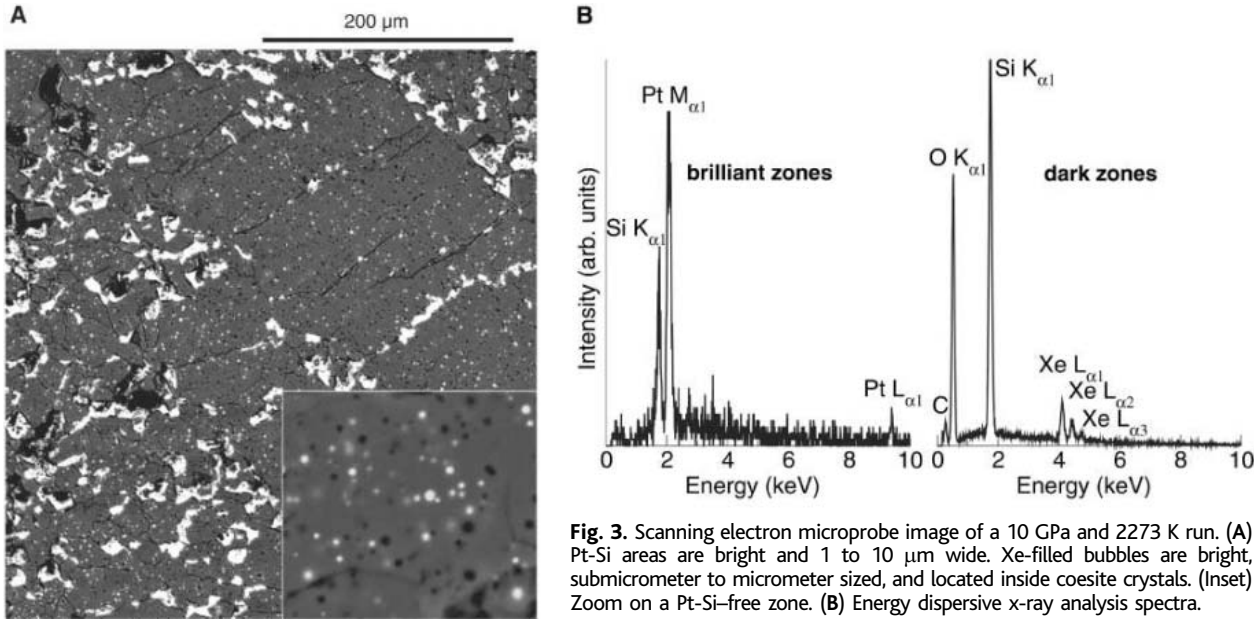


Fig. 3. Scanning electron microprobe image of a 10 GPa and 2273 K run. (**A**) Pt-Si areas are bright and 1 to 10 μm wide. Xe-filled bubbles are bright, submicrometer to micrometer sized, and located inside coesite crystals. (Inset) Zoom on a Pt-Si–free zone. (**B**) Energy dispersive x-ray analysis spectra.

consistent with the peaks observed upon temperature quenching, which can be assigned to a monoclinic cell (a = 7.66 Å, b = 7.76 Å, c = 7.78 Å, β = 88.27°, Fig. 1). The presence of a Pt-Si compound is also consistent with the appearance of a liquid above 1100 K (Fig. 1), which is the eutectic temperature in the Pt-Pt_2Si system at ambient pressure (*18*). Silica was therefore reduced and Si released, and as a consequence, the melting point of Pt was lowered.

The sample that was recovered from a multi-anvil run carried out at 10 GPa and 2273 K was also a mixture of Pt-Si zones and coesite crystals rich in Xe bubbles (Fig. 3), revealing the same reduction process of silica. The XeO_4 molecule, as observed by chemical synthesis, has a tetrahedral structure (*19*) in which the Xe-O distance is 1.736 Å at ambient conditions in the gas phase. On the other hand, the Si-O distance in either α- or β-quartz tetrahedra is close to 1.6 Å for the investigated P and T range and close to 1.55 Å in coesite at 5 GPa. The Raman signature of XeO_4 (*20*) tetrahedra presents two bands that could correspond to the peaks observed, in addition to a quartz signal at 356 and 814 cm^{-1} in high P and T Raman spectroscopic studies of the SiO_2-Xe system (*21*, *22*).

We therefore propose that the exsolution of Si from either quartz or coesite occurs through substitution of Si by Xe in the tetrahedral network at high temperature. This reaction is accompanied by an important volume reduction, mostly because it takes place at temperatures above the melting curve of Xe, implying a large Xe molar volume (*23*). This volume reduction contributes negatively to the free enthalpy, e.g., $P\Delta V$ = –700 kJ/mol at 5 GPa and 1500 K, where V is volume, favoring the reaction that is otherwise inhibited at ambient conditions because of the high formation enthalpy predicted for XeO_2 (*24*) compared with that of SiO_2.

At room temperature, Xe diffuses out, which is indicated by the presence of solid Xe diffraction peaks in the present study (Fig. 1) and by the disappearance of the extra Raman bands (*21*). This retrodiffusion results in a mean concentration of Xe in quartz of 2.2 weight percent (wt%) in quenched samples, whereas 30 to 40 wt% was initially loaded (*15*) and had fully reacted. This remaining Xe was trapped mostly in micrometer- to submicrometer-sized bubbles (Fig. 3). Xe content was below the detection threshold in magnesiowüstite + Xe samples. Because no modification of either Xe or magnesiowüstite phase diagrams was observed in x-ray diffraction data, we conclude that both components do not interact. Quenched α-quartz in our experiments presents the following peculiarities compared with normal α-quartz: Cell parameters are slightly smaller; but, more markedly, Raman spectra show a significant and variable shift of certain modes (fig. S1) (*15*), whereas the frequencies of coesite modes when present are normal. This finding could be related to the presence of defects attributed to Xe leaving the $(Si,Xe)O_2$ network and creating vacancies.

Data on Xe concentrations in continental crustal rocks are scarce. A few occurrences of rocks with high Xe content have been reported, for instance in a granite from the Sudbury impact crater (*25*) or for siliceous sediments (*6*). Xe could become trapped in crustal silica in three different ways: (i) during early heavy meteoritic bombardements, (ii) indirectly by adsorption of Xe on siliceous sediments and further processing into granites or granulites, and (iii) locally upon radiogenic production. The first scenario is the only one to provide an explanation for the depletion of light Xe isotopes in the atmosphere through kinetic processes. Alternatively, this depletion in light isotopes might not be related to the missing Xe problem but rather may be the result of early hydrodynamic escape (*8*).

The timing of atmospheric Xe loss can be estimated to about 100 million years (My) from accretion, given the isotope abundances of radiogenic Xe produced by ^{129}I and ^{244}Pu (*26*, *27*). This timing constrains the missing Xe problem: Xe cannot be hidden in a reservoir that formed either on a too-long time scale (e.g., sediments), or a too-short time scale (e.g., the core). Core formation was likely completed within 30 My of planetary accretion (*28*). The continental crust is a good candidate in this respect. A major mantle differentiation event likely occurred within the first 100 to 150 My of accretion, as deducted from $^{146}Sm/^{142}Nd$ systematics (*29*, *30*).

References and Notes

1. E. Anders, T. Owen, *Science* **198**, 453 (1977).
2. J. Kunz, T. Staudacher, C. J. Allegre, *Science* **280**, 877 (1998).
3. W. A. Caldwell *et al.*, *Science* **277**, 930 (1997).
4. J. F. Wacker, E. Anders, *Geochim. Cosmochim. Acta* **48**, 2373 (1984).
5. G. T. Sill, L. L. Wilkening, *Icarus* **33**, 13 (1978).
6. J.-I. Matsuda, K. Matsubara, *Geophys. Res. Lett.* **16**, 81 (1989).
7. D. Krummenacher, C. M. Merrihue, R. O. Pepin, J. H. Reynolds, *Geochim. Cosmochim. Acta* **26**, 231 (1962).
8. R. O. Pepin, *Icarus* **92**, 2 (1991).
9. A. P. Jephcoat, *Nature* **393**, 355 (1998).
10. E. M. Chamorro-Perez, P. Gillet, A. Jambon, *Earth Planet. Sci. Lett.* **145**, 97 (1996).
11. B. Schmidt, H. Keppler, *Earth Planet. Sci. Lett.* **195**, 277 (2002).
12. R. A. Brooker *et al.*, *Nature* **423**, 738 (2003).
13. M. Pettersson, J. Lundell, M. Räsänen, *Eur. J. Inorg. Chem.* **505**, 729 (1999).
14. C. Sanloup, H.-K. Mao, R. J. Hemley, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 25 (2002).
15. Materials and methods are available as supporting material on *Science* Online.
16. M. C. Bellissent-Funel, U. Buontempo, A. Filabozzi, C. Petrillo, F. P. Ricci, *Phys. Rev. B* **45**, 4605 (1992).
17. J. P. Périllat, I. Daniel, J. M. Lardeaux, H. Cardon, *J. Petrol.* **44**, 773 (2003).
18. M. Hansen, *Constitution of Binary Alloys* (McGraw-Hill, New York, 1958).
19. G. Gundersen, K. Hedberg, *J. Chem. Phys.* **52**, 812 (1970).

20. M. Gerken, G. J. Schrobilgen, *Inorg. Chem.* **41**, 198 (2002).
21. C. Sanloup, R. J. Hemley, H.-K. Mao, *Geophys. Res. Lett.* **29**, 30 (2002).
22. In Raman spectroscopy experiments (*21*), a third peak was also observed in addition to the quartz signal at 588 cm^{-1}. This position cannot be reconciled with the Si band, 520 cm^{-1} at ambient conditions, neither can it be with any silica phase. We propose instead that it corresponds to the main Raman band of SiO units, located at 596.4 cm^{-1} at room temperature in the gas phase (*31*), as a result of the following reaction

$$Si + SiO_2 \Leftrightarrow 2\ SiO.$$

23. O. Sifner, J. Klomfar, *J. Phys. Chem. Ref. Data* **23**, 63 (1994).
24. P. Pykkö, T. Tamm, *J. Phys. Chem. A* **104**, 3826 (2000).
25. P. K. Kuroda, R. D. Sherrill, in *Abundances and Isotopic Compositions of Rare Gases in Granites and Thucholites* (Center for Academic Publications, Tokyo, Japan, 1978), pp. 63–64.
26. G. W. Wetherill, *Annu. Rev. Nucl. Sci.* **25**, 283 (1975).
27. M. Ozima, F. A. Podosek, *J. Geophys. Res.* **104**, 25493 (1999).
28. T. Kleine, C. Münker, K. Mezger, H. Palme, *Nature* **418**, 952 (2002).
29. M. Boyet *et al.*, *Earth Planet. Sci. Lett.* **214**, 427 (2003).
30. G. Caro, B. Bourdon, J.-L. Birck, S. Moorbath, *Nature* **423**, 428 (2003).
31. M. Friesen, M. Junker, A. Zumbusch, H. Schnöeckel, *J. Chem. Phys.* **111**, 7881 (1999).
32. We thank A. Dewaele, B. Marty, and M. Moreira for discussions; M. Fialin, G. Montagnac, and O. Boudouma for analytical support; and two anonymous reviewers for their constructive comments that greatly benefited the paper. This research was supported by the Access to Large Facilities European program (Bayerisches Geo-Institut), the French Programme National de Planétologie, and the European Synchrotron Radiation Facility.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5751/1174/DC1
Materials and Methods
Fig. S1
References

18 August 2005; accepted 12 October 2005
10.1126/science.1119070

Dinosaur Coprolites and the Early Evolution of Grasses and Grazers

Vandana Prasad,[1] Caroline A. E. Strömberg,[2]* Habib Alimohammadian,[3] Ashok Sahni[3]

Silicified plant tissues (phytoliths) preserved in Late Cretaceous coprolites from India show that at least five taxa from extant grass (Poaceae) subclades were present on the Indian subcontinent during the latest Cretaceous. This taxonomic diversity suggests that crown-group Poaceae had diversified and spread in Gondwana before India became geographically isolated. Other phytoliths extracted from the coprolites (from dicotyledons, conifers, and palms) suggest that the suspected dung producers (titanosaur sauropods) fed indiscriminately on a wide range of plants. These data also make plausible the hypothesis that gondwanatherian mammals with hypsodont cheek teeth were grazers.

Today, grasses (in the family Poaceae) are extant on all continents except Antarctica, and numerous organisms, not least humans, depend on them for food (such as cereals and feed for domesticated animals) and habitat (*1*). The absence of an early fossil record of grasses has prevented detailed examination of their evolution and coevolution with animals. Presumed grass pollen (*Monoporites*) in the Maastrichtian to Paleocene [70 to 60 million years ago (Ma)] of South America, India, and North Africa marks the earliest fossil record of Poaceae (*1*, *2*), and unequivocal macrofossils of crown-group Poaceae appear no earlier than the Late Paleocene (~55 Ma) (*2*, *3*). Rare macrofossils and a phytolith record from North America point to diversification of the two main grass subclades, BEP (Bambusoideae + Ehrhartoideae + Pooideae) and PACCAD (Panicoideae + Arundinoideae + Chloridoideae + Centothecoideae + Aristidoideae + Danthonioideae), in the Late Eocene (~35 Ma) (*4*). In contrast, recent molecular clock estimates provide substantially older dates for the origin of both crown-group Poaceae (~83 Ma) (*5*) and of the BEP and PACCAD subclades (~55 Ma) (*3*). Despite these discrepancies, molecules and fossils both support a

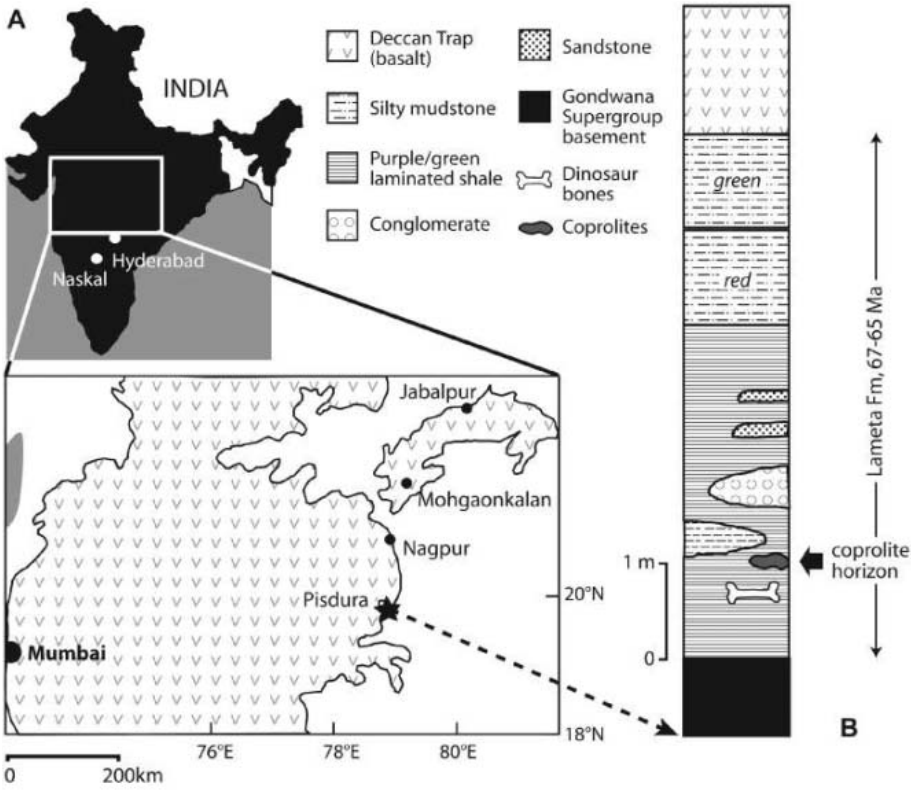


Fig. 1. (**A**) Geographic location of the coprolite site at Pisdura in the Deccan Traps, India. Grass macrofossils have been found at Mohgaonkalan (*18*–*20*), and an unnamed gondwanatherian mammal has been described from Naskal (small map at top left) (*8*). (**B**) Stratigraphic position of the coprolite horizon in the Lameta Formation in the measured section at Pisdura. The Maastrichtian age of the Intertrappean beds at Pisdura is based on biostratigraphical, magnetostratigraphical, and geochronological data from this and correlated sections (*13*, *14*, *29*–*31*).

predominantly Cenozoic radiation of the grass clade (*3*). The biogeographical history of grasses is equally obscure. However, the distribution of extant Poaceae taxa point to a South American origin for the family (*3*), an inference that is supported by the aforementioned earliest occurrences of grass pollen (*2*). Current molecular dating and fossil data (*3*, *5*) also favor long-distance dispersal of all major Poaceae lineages after the Late Cretaceous breakup of Gondwana.

Because grasses were thought to have been rare in pre-Cenozoic ecosystems, they have not been considered as food for Late Cretaceous herbivores (*2*, *6*). Titanosaur sauropods, the most prominent terrestrial plant-eaters in Gondwana, do not display any dental features (such as grinding cheek teeth) that would point to grass-eating (*6*, *7*). Mammals with typical grazing adaptations, particularly high-crowned (hypsodont) teeth, occur mainly during the Oligocene and Miocene, indicating that grasses were not previously present in sufficient abundance to form major parts of an herbivorous diet (*2*). A striking exception are the enigmatic sudamericid gondwanatherians, known from the Late Cretaceous of South America, Madagascar, possibly Tanzania, and India, as well as the Paleogene of South America and Antarctica [reviewed in (*8*–*10*)]. Their highly hypsodont teeth appear decidedly suited for handling abrasive materials, such as grass (*11*). However, because of the lack of contemporary grass fossils, they have been interpreted as an adaptation to a semiaquatic or burrowing lifestyle, reminiscent of that of modern beavers (*11*).

Here we provide fossil data on Late Cretaceous grasses that shed light on the early evolution of grasses and grass-eaters. The grass fossils, in the form of phytoliths, are preserved in coprolites from the Late Cretaceous (Maastrichtian) Intertrappean beds of the fluvio-lacustrine Lameta Formation, at Pisdura in central India (Fig. 1). The coprolites are found in abundance weathering out on surface exposures (*12*). The four types (A, B, Ba, and C) of coprolites have all been ascribed to titanosaur sauropods, based on their common association with titanosaur skeletal remains (*13*–*15*). Previous work on type A coprolites shows that they contain remains of predominantly C_3 plants, including conifers and cycads, but also bacterial colonies, fungal spores, and algal remains (*14*, *15*).

We extracted bio-opal (phytoliths, diatoms, etc.) from all types (A, B, Ba, and C) of coprolites (*16*). The recovered organic and silicified plant fossils include cells and tissue from what were likely (i) dicotyledons [polyhedral and anticlinal epidermis, often with vesicular infillings, helical tracheary elements, honeycomb mesophyll, cystoliths, and nongrass trichomes (*17*)]; (ii) large pieces of silicified parenchymatous tissue probably referable to conifers; (iii) rare globular echinate phytoliths diagnostic of palms; and (iv) numerous silica phytoliths of certain-to-likely grass origin. The range of grass morphotypes is similar to that found in phytolith assemblages extracted from the leaves of modern grasses. To taxonomically place the fossil phytoliths, we collected information on Poaceae epidermal anatomy and grass silica short cell (GSSC) morphology from the literature and a phytolith reference collection (table S1) (*17*) and mapped these data onto a cladogram of the grass family (*1*) (fig. S1).

The phytolith data from the coprolites reveal a diversity of phytolith morphotypes (expressed as new phytolith morphotype genera and species; see supporting online text), suggesting that several grass taxa representing different Poaceae subclades were extant in India during the Late Cretaceous (Figs. 2 and 3). These include: (i) a derived ehrhartoid (*Matleyites indium*), (ii) possibly another grass within the [Bambusoideae + Ehrhartoideae]

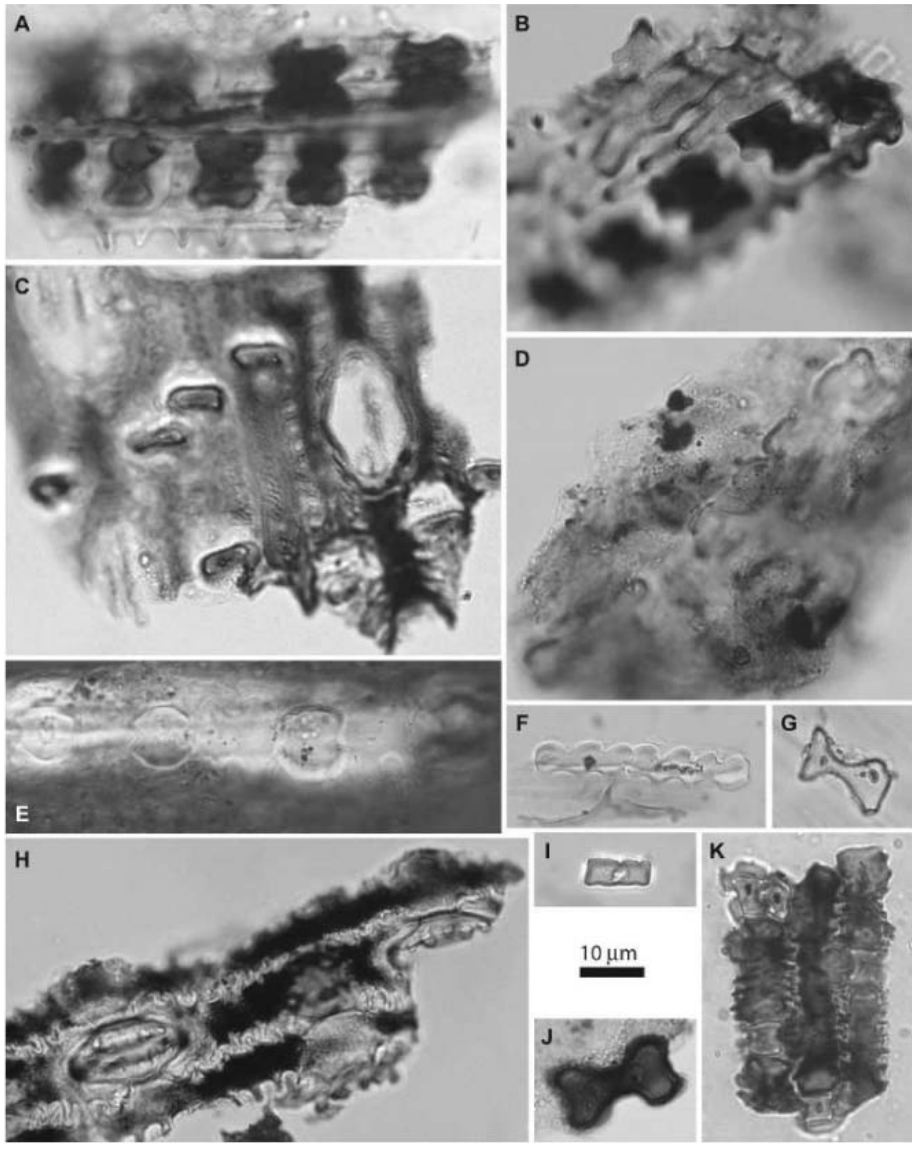


Fig. 2. Selected fossil phytolith morphotypes described here. (**A** and **B**) *Matleyites indium*. Costal row(s) of vertical "oryzoid" bilobate and cross-shaped GSSCs with pointed lobes associated with papillate epidermis. (**C**) *Jainium pisdurensis*. Intercostal epidermis with stomates and vertical chusquoid/bilobate GSSCs. (**D**) *Chitaleya deccana*. Costal row (obliquely across the photo) of three symmetrical bilobate and polylobate GSSCs with concave ends. (**E**) *Pipernoa pearsalla*. Costal row of collapsed saddles. (**F**) *Chitaleya deccana*. Two polylobate GSSCs in a row. (**G**) *Eliasundo lameti*. Isolated tabular angular bilobate GSSC. (**H**) *Vonhueneites papillosum*. Fusiform stomates with papillate subsidiary cells associated with papillate epidermal long cells. (**I**) *Thomassonites sinuatum*. Isolated trapeziform sinuate ("crenate"). (**J**) *Matleyites indium*? Isolated bilobate GSSC with pointed lobes. (**K**) *Stebbinsana intertrappea*. Epidermis with horizontal trapezoidal bilobate GSSCs with concave top. A light microscope was used to take these images [in (E), with a Nomarski filter].

[1]Birbal Sahni Institute of Palaeobotany, Lucknow-226 007, India. [2]Departments of Palaeobotany and Palaeozoology, Swedish Museum of Natural History, SE-104 05 Stockholm, Sweden. [3]Centre of Advanced Study in Geology, Panjab University, Chandigarh-160014, India.

*To whom correspondence should be addressed. E-mail: caroline.stromberg@nrm.se

clade (*Vonhueneites papillosum*), (iii) a grass within the [Bambusoideae + Ehrhartoideae] clade or the Puelioideae (*Pipernoa pearsalla*), (iv) a grass of the PACCAD clade or with affinity to Pooideae (*Chitaleya deccana*), (v) an ingroup pooid (or PACCAD grass) (*Thomassonites sinuatum*), and (vi) another grass with affinity to PACCADs (*Eliasundo lameti*). Several other grass morphotypes (*Jainium pisdurensis* and *Stebbinsana intertrappea*) attest to the morphological diversity of GSSCs, but their systematic position within Poaceae cannot be ascertained. Although some of the phytolith morphotypes may derive from the same grass, a minimum of five different taxa from the [Bambusoideae + Ehrhartoideae] clade and the PACCAD or Pooideae clades are represented (supporting online text). Thus, most of the morphotypes observed appear to be more derived than the basal-most grass taxa (*Anomochloa*, *Streptochaeta*, and *Pharus*), based on GSSC morphology and the assumption that these ingroup taxa can confidently be used to inform plesiomorphic character states within Poaceae. The existence of crown-group Poaceae in Late Cretaceous India is consistent with the presence there of Intertrappean macrofossils described as pooids (*18*–*20*) (Fig. 1) and the occurrence of grass pollen (*Graminidites*) in the Lameta Formation of the Nand-Dongargaon Area (*21*).

The presence of ehrhartoids and possible ingroup pooids in the Late Cretaceous shows that the BEP clade had diversified substantially by this time (Fig. 3), much earlier than had been thought on the basis of fossils and molecular clock dating (*1*, *3*, *4*). An early radiation of both the Pooideae and the PACCAD clade is also suggested by the occurrence of GSSCs typical of pooids and PACCAD grasses, respectively. It is not yet clear whether the ecomorphologies that allow extant pooids and PACCADs to inhabit open, arid habitats had evolved by the Late Cretaceous.

The hypothesis that extant grass subgroups arose in Asia and subsequently spread to Gondwana by way of India by the Late Cretaceous is highly improbable in light of the data pointing to a collision between the Indian subcontinent and Asia in the Paleogene (55 to 50 Ma), with no compelling support for an earlier connection (*22*, *23*). The diversification of the BEP and PACCAD clades could have occurred on the Indian subcontinent, after which grass taxa dispersed globally via Asia and North America, starting in the Paleocene. This option is unlikely, given the paucity of grass pollen in Paleocene-Eocene floras of the Northern Hemisphere as compared to the Southern Hemisphere (*2*). Our preferred hypothesis is that the various Poaceae subclades had spread in Gondwana by the Late Cretaceous, as a result of either vicariance or dispersal [for example, via the Kerguelen Plateau or the Gunnerus Ridge (*24*, *25*)]. This scenario does not necessitate a Gondwanan origin of Poaceae or any of its subclades; the initial diversification of extant lineages could have occurred in India, in another Gondwanan continent, or in the Northern Hemisphere [compare to discussion in (*10*)]. However, it postulates that BEP and PACCAD grasses ranged across at least part of Gondwana by the time India lost its biogeographic connections with other southern continents; that is, by ~80 Ma (*22*, *24*, *25*).

The examined phytoliths show variable preservation, implying that some phytoliths (such as large pieces of tissue) were incorporated into the coprolites as part of foodstuffs, whereas others (such as rare palm phytoliths) may have derived from soil stuck to ingested plant material. Previous studies of coprolite and enterolite material from Late Cretaceous herbivorous dinosaurs have suggested that these animals fed mainly on conifers but also consumed cycads and angiosperms (*14*, *26*, *27*). Phytolith data show that their diet must have been very mixed, including conifers, cycads (*14*), dicotyledons, and grasses. Because phytolith production in extant grasses is magnitudes higher than in dicotyledons and conifers, the relatively low abundance of grass phytoliths in the coprolites may indicate that grasses did not form a major part of the animals' diet.

There is as yet no direct evidence for mammalian grazing in the Late Cretaceous. However, the presence of both diverse silica-rich grasses and sudamericid gondwanatherians in

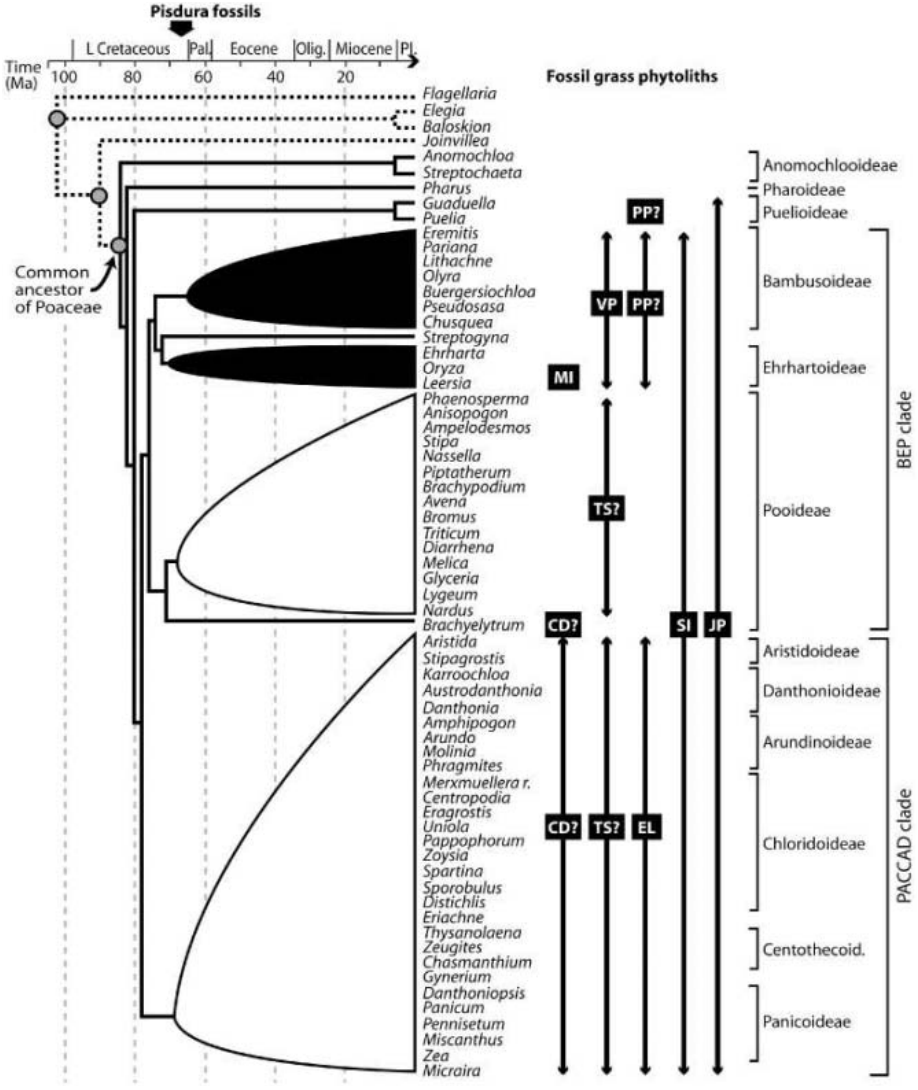


Fig. 3. Systematic affinities of fossil phytolith morphotypes (Supporting Online Text) reported from Maastrichtian coprolites from Pisdura, central India, suggesting significantly older dates for taxonomic diversification within the grass family (Poaceae) than previously assumed (*1*–*3*). The phylogeny is from the Grass Phylogeny Working Group (*1*). Approximate ages for the crown node of Poaceae and for immediate sister taxa (marked with a gray circle) were provided by molecular clock analysis (*5*). White shapes indicate open-habitat grass clades; black shapes and all other terminal taxa indicate closed-habitat grasses. Fossil phytolith morphotypes are as follows: CD, *Chitaleya deccana*; EL, *Eliasundo lameti*; JP, *Jainium pisdurensis*; MI, *Matleyites indium*; PP, *Pipernoa pearsalla*; SI, *Stebbinsana intertrappea*; TS, *Thomassonites sinuatum*; VP, *Vonhueneites papillosum*.

the Intertrappean beds of India makes it plausible that hypsodonty in these animals was an adaptation to feeding on abrasive grasses. Moreover, the phytolith data suggest that silica production in grasses comparable with that observed in extant taxa appeared to have evolved by the Late Cretaceous. This rejects the view that modern levels of phytolith production were an evolutionary response to grazing during the Cenozoic (*28*) and suggests that the high silica levels of grasses are the result of coevolution with Late Cretaceous herbivores (such as gondwanatherians or insects) or of a process unrelated to plant/herbivore interaction.

References and Notes

1. Grass Phylogeny Working Group, *Ann. Mo. Bot. Gard.* **88**, 373 (2001).
2. B. F. Jacobs, J. D. Kingston, L. L. Jacobs, *Ann. Mo. Bot. Gard.* **86**, 590 (1999).
3. K. Bremer, *Evolution* **56**, 1374 (2002).
4. C. A. E. Strömberg, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11980 (2005).
5. T. Janssen, K. Bremer, *Bot. J. Linn. Soc.* **146**, 385 (2004).
6. P. M. Barrett, K. J. Willis, *Biol. Rev. Camb. Philos. Soc.* **76**, 411 (2001).
7. J. A. Wilson, P. C. Sereno, *Mem. Soc. Vertebr. Paleontol.* **5**, 1 (1998).
8. D. W. Krause, G. V. R. Prasad, W. von Koenigswald, A. Sahni, F. E. Grine, *Nature* **390**, 504 (1997).
9. D. W. Krause, M. D. Gottfried, P. M. O'Connor, E. M. Roberts, *Acta Palaeontol. Pol.* **48**, 321 (2003).
10. R. S. Rana, G. P. Wilson, *Acta Palaeontol. Pol.* **48**, 331 (2003).
11. R. Patnaik, A. Sahni, G. V. R. Prasad, *J. Palaeontol. Soc. India* **46**, 15 (2001).
12. C. A. Matley, *Rec. Geol. Surv. India* **74**, 535 (1939).
13. F. B. Von Huene, C. A. Matley, *Mem. Geol. Surv. India* **21**, 1 (1933).
14. P. Ghosh *et al.*, *Cretaceous Res.* **24**, 743 (2003).
15. N. Sharma, R. K. Kar, A. Agarwal, R. Kar, *Micropaleontology* **51**, 73 (2005).
16. Materials and methods are available as supporting material on *Science* Online.
17. C. A. E. Strömberg, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **207**, 239 (2004).
18. S. D. Chitaley, M. T. Sheikh, *J. Indian Bot. Soc.* **50**, 137 (1971).
19. G. V. Patil, R. B. Singh, *A. K. Ghosh Commemoration Volume*, A. K. Sharma, G. C. Mitra, M. Banerjee, Eds. (Today and Tomorrow's Printers & Publishers, New Delhi, India, 1984), pp. 89–96.
20. B. S. Trivedi, S. K. Bajpai, *J. Indian Bot. Soc.* **61**, 320 (1982).
21. B. Samant, D. M. Mohabey, paper presented at the XIX Indian Colloquium on Micropalaeontology and Stratigraphy, Symposium on Recent Developments in Indian Ocean Palaeoceanography and Palaeoclimate, Varanasi, India, 9 to 11 October 2003.
22. S. McLoughlin, *Aust. J. Bot.* **49**, 271 (2001).
23. W. C. Clyde, I. H. Khan, P. D. Gingerich, *Geology* **31**, 1097 (2003).
24. W. W. Hay *et al.*, in *Evolution of the Cretaceous Ocean–Climate System*, E. Barrera, C. C. Johnson, Eds. (Geological Society of America, Boulder, CO, 1999), pp. 1–47.
25. J. A. Case, *J. Vertebr. Paleontol.* **22**, 42A (2002).
26. K. Chin, B. D. Gill, *Palaios* **11**, 280 (1996).
27. R. A. Rodríguez de la Rosa, S. R. S. Cevallos-Ferriz, A. Silva-Pineda, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **142**, 231 (1998).
28. G. L. Stebbins, *Ann. Mo. Bot. Gard.* **68**, 75 (1981).
29. V. Courtillot *et al.*, *Nature* **333**, 843 (1988).
30. C. K. Khajuria, G. V. R. Prasad, B. K. Manhas, *Newsl. Stratigr.* **31**, 21 (1994).
31. M. Widdowson, M. S. Pringle, O. A. Fernandez, *J. Petrol.* **41**, 1177 (2000).
32. We thank N. C. Mehrotra for facilities and support, and S. Kad and K. Ambwani for help. C.A.E.S. is supported by a grant to E. M. Friis and L. Werdelin at the Swedish Museum of Natural History, Stockholm. We thank J. A. Wilson, G. P. Wilson, and two anonymous reviewers for helpful comments and suggestions.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1177/DC1
Materials and Methods
SOM Text
Fig. S1
Table S1
References

12 August 2005; accepted 12 October 2005
10.1126/science.1118806

Pre- and Postinvasion Defenses Both Contribute to Nonhost Resistance in *Arabidopsis*

Volker Lipka,[1]*† Jan Dittgen,[1]* Pawel Bednarek,[1] Riyaz Bhat,[1] Marcel Wiermer,[1] Monica Stein,[2] Jörn Landtag,[3] Wolfgang Brandt,[4] Sabine Rosahl,[3] Dierk Scheel,[3] Francisco Llorente,[5] Antonio Molina,[5] Jane Parker,[1] Shauna Somerville,[2] Paul Schulze-Lefert[1]‡

Nonhost resistance describes the immunity of an entire plant species against nonadapted pathogen species. We report that *Arabidopsis* PEN2 restricts pathogen entry of two ascomycete powdery mildew fungi that in nature colonize grass and pea species. The PEN2 glycosyl hydrolase localizes to peroxisomes and acts as a component of an inducible preinvasion resistance mechanism. Postinvasion fungal growth is blocked by a separate resistance layer requiring the EDS1-PAD4-SAG101 signaling complex, which is known to function in basal and resistance (*R*) gene–triggered immunity. Concurrent impairment of pre- and postinvasion resistance renders *Arabidopsis* a host for both nonadapted fungi.

Host cell entry represents a critical step during pathogenesis of invasive animal and plant parasites (*1*, *2*). This is usually not a barrier to adapted phytopathogenic fungi that are able to infect a plant species. However, in plant species beyond the host range of a pathogen, called nonhost plants, parasitic fungi typically fail to enter attacked plant cells (*3*, *4*). In the nonhost interaction between *Arabidopsis* and the grass powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*), three *Arabidopsis pen* (penetration) mutant loci were recovered that permit, at high frequency, entry of the nonadapted parasite (*5*), thereby providing initial evidence for the existence of a plant-controlled process terminating fungal ingress at the cell periphery. *AtPEN1* encodes a soluble NSF (*N*-ethylmaleimide–sensitive factor) attachment protein receptor (SNARE)–domain-containing and plasma-membrane resident syntaxin, which becomes recruited into plasma membrane microdomains beneath incipient fungal entry sites (*6*, *7*). Because SNARE proteins play a key role in vesicle trafficking in eukaryotic cells (*8*), these findings have been interpreted as evidence for the existence of a vesicle-associated resistance mechanism preventing powdery mildew ingress.

Although each of the isolated *pen* mutants (*pen1*, *pen2*, and *pen3*) permits efficient *Bgh* entry, initiation of postinvasive fungal growth invariably ceases, and this coincides with a cell death response of epidermal cells with haustorial complexes (*5*). We performed a time-course experiment and compared *Bgh* entry rates in wild type, *pen1*-1, *pen2*-1, and *pen1 pen2* double null mutants (Fig. 1). Entry rates were seven- and fivefold higher than wild type in *pen1* and *pen2* mutants, respectively. An 11-fold increase over wild type was seen in the *pen1 pen2* genotype, suggesting *PEN1* and *PEN2* act in separate defense pathways (Fig. 1). Elevated fungal entry rates were associated with an increased incidence of invasion-associated cell death (Fig. 1). To assess the importance of *PEN1* and *PEN2* functions in a further powdery mildew nonhost interaction, we examined the mutant *Arabidopsis* genotypes with *Erysiphe pisi*, which colonizes dicotyledonous pea plants in nature. Like *Bgh*, *E. pisi* fails to reproduce on *Arabidopsis* but

[1]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Carl von Linné Weg 10, D-50829 Köln, Germany. [2]Department of Plant Biology, Carnegie Institute of Washington, Stanford, CA 94305, USA. [3]Department of Stress and Developmental Biology, [4]Department of Bioorganic Chemistry, Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany. [5]Laboratorio de Bioquímica y Biología Molecular, Departamento de Biotecnología-Universidad Politécnica de Madrid Escuela Técnica Superior de Ingenieros Agrónomos, Avenida Complutense s/n, E-28040 Madrid, Spain.

*These authors contributed equally to this work.
†Present address: Zentrum für Molecularbiologie der Pflazen, Plant Biochemistry, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 5, D-72076 Tübingen, Germany.
‡To whom correspondence should be addressed. E-mail: schlef@mpiz-koeln.mpg.de

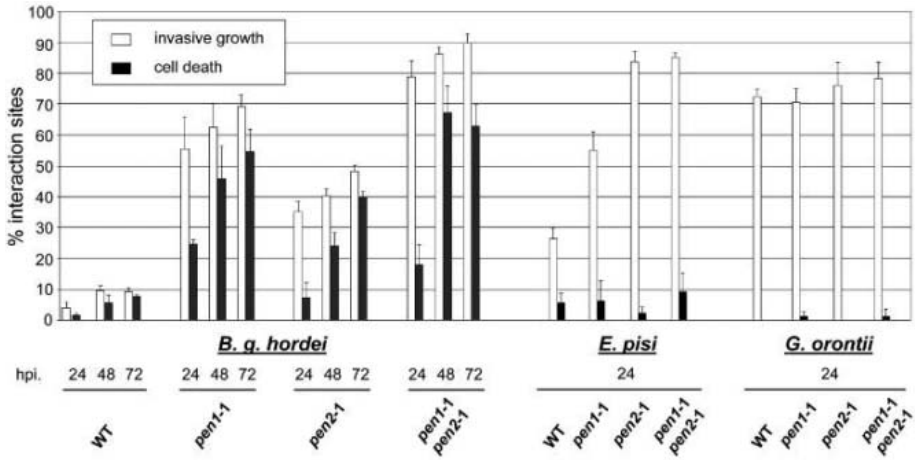


Fig. 1. Frequency of invasive growth and epidermal single-cell death at *B. g. hordei*, *E. pisi*, and *G. orontii* interaction sites on *Arabidopsis* wild type (WT) and *pen1*-1, *pen2*-1 and *pen1*-1 *pen2*-1 mutants. hpi, hours post–conidiospore inoculation. Error bars indicate standard deviations for triplicate measurements.

is phylogenetically more closely related to the *Arabidopsis*-infecting powdery mildew *E. cruciferarum* than to *Bgh* (*9*). Absence of PEN1 or PEN2 greatly enhanced the entry rates of *E. pisi*. However, *E. pisi* successfully invaded wild-type *Arabidopsis* more often than *Bgh* [~25% versus ~5% at 24 hours postinoculation (Fig. 1)], and lack of PEN2 alone permitted an *E. pisi* entry frequency similar to those of the *pen1 pen2* double mutants (Fig. 1). Invasiveness of *Bgh* on *pen1 pen2* double mutants and of *E. pisi* on *pen2* or *pen1 pen2* plants is essentially indistinguishable from that of the adapted *Golovinomyces orontii* powdery mildew on wild-type *Arabidopsis* (70 to 80%) (Fig. 1). Invasiveness of *G. orontii* does not increase on either *pen* mutant genotype, indicating that PEN1 and PEN2 exert entry-limiting functions only in nonhost powdery mildew interactions.

A difference between *pen1* and *pen2* plants was revealed after inoculation with the nonadapted hemibiotrophic oomycete *Phytophthora infestans*. Only *pen2* mutants allowed frequent initiation of invasive growth of this potato pathogen (fig. S1A). Similar to invasion-associated cell death in interactions with the inappropriate powdery mildew species (Fig. 1 and fig. S1B), invasive growth of the oomycete in *pen2* plants is linked to a localized plant cell death response (fig. S1C). Likewise, inoculation with the broad host range ascomycete *Plectosphaerella cucumerina* resulted in enhanced disease susceptibility only in *pen2* mutants (fig. S1D). Thus, PEN2 limits growth of a wide spectrum of pathogens, whereas PEN1 function is limited to nonadapted powdery mildew species.

PEN2 was isolated by map-based cloning (fig. S2A) and encodes 1 out of 48 predicted *Arabidopsis* family 1 glycosyl hydrolases (F1GHs) (fig. S2B). Both chemically induced *pen2* mutant alleles, *pen2*-1 and *pen2*-3, are characterized by point mutations, each generating stop codons that lead to truncated peptides (fig. S2, B and C). We also isolated a homozygous transfer DNA (T-DNA) insertion line resulting in loss of detectable PEN2 transcript and protein (designated *pen2*-2; fig. S2, A to C). The infection phenotype of *pen2*-2 plants is indistinguishable from the two chemically induced mutants. F1GHs are present in all eukaryotic and prokaryotic organisms and hydrolyze O or S glycosidic bonds between two or more carbohydrates or between a carbohydrate and a noncarbohydrate (i.e., aglycone). In plants, F1GHs have been implicated in a wide range of processes, including development, cell wall modification, and chemical defense against pathogens (*10*).

Cyanogenic β-glucosidase CBG1 from *Trifolium repens* shares 48% sequence identity (65% similarity) with PEN2 (fig. S2B). This enabled us to use the known three-dimensional structure of CBG1 as template for homology modeling of a PEN2 structure (Fig. 2A and Materials and Methods). The topview ribbon diagram of PEN2 shows the typical F1GH barrel fold structure with a central cleft gate to the active site, which harbors the catalytic machinery consisting of two conserved acid/base and nucleophilic glutamates (Glu^{183} and Glu^{398}, denoted by E183 and E398, respectively), located at the bottom of the active site pocket (Fig. 2A) (*11*). To test whether PEN2 catalytic activity is required for pathogen entry control, we generated transgenic lines in a *pen2*-1 mutant background, expressing a $Glu^{183} \rightarrow Asp^{183}$ (E183D) substitution variant of PEN2 ($PEN2_{E183D}$) under the control of native *PEN2* 5′ regulatory sequences (fig. S2D). Unlike control transgenic lines expressing wild-type PEN2, none of the tested $PEN2_{E183D}$ lines complemented the *pen2* mutant phenotype, although a PEN2-specific antiserum detected comparable $PEN2_{E183D}$ protein amounts in leaf extracts (Fig. 2B). This suggests that PEN2 catalytic activity is required to restrict pathogen entry.

An unusual feature of the PEN2 F1GH is the presence of a C-terminal extension containing a low-complexity region of 15 amino acids followed by a predicted helical region of 28 amino acids (Fig. 2A and fig. S2B). To test a potential functional contribution of the C-terminal extension, we generated transgenic lines in the *pen2*-1 background expressing a truncated PEN2 protein that lacked the C-terminal 28 amino acids ($PEN2_{\Delta 28}$; fig. S2, B and D). Although the truncation did not affect protein stability (Fig. 2B), $PEN2_{\Delta 28}$ failed to complement the *pen2* phenotype upon *Bgh* or *E. pisi* challenge, indicating that the C-terminal extension may regulate enzyme activity and/or serve as a determinant for subcellular localization.

To examine PEN2 localization in living cells, we generated transgenic lines expressing various PEN2–green fluorescent protein (GFP) fusion constructs driven by native 5′ regulatory *PEN2* sequences in the *pen2*-1 null mutant background. Because both N- and C-terminal fusions of PEN2 to GFP were found to produce nonfunctional proteins, we searched for candidate GFP insertion sites in PEN2 on the basis of the deduced structural model (Fig. 2A). Indeed, insertion of GFP between the predicted globular PEN2 enzyme and the C-terminal extension resulted in a fluorescent fusion protein that complemented the *pen2* phenotype. Confocal imaging revealed a distinctive subcellular GFP fluorescence pattern, indicating association of PEN2 to mobile vesicle-like bodies (Fig. 2C). Coexpression of fluorophore-tagged marker proteins for the Golgi, mitochondria, or peroxisomes identified these as peroxisomes in time-lapse imaging experiments (fig. S3A), which is in agreement with the PEN2 subcellular localization predicted by PSORT (*12*). The two-color imaging experiments showed the expected matrix localization for red fluorescent protein (RFP) containing a type 1 peroxisomal targeting sequence, whereas PEN2-GFP appeared to be confined to the periphery of peroxisomes (fig. S3A). A striking focal accumulation of PEN2-GFP-tagged peroxisomes was seen upon inoculation with *Bgh* conidiospores at incipient entry sites (14 to 16 hours postinoculation) (Fig. 2D). This pathogen-inducible PEN2 accumulation at fungal entry sites coincides with its predicted biological function at the cell periphery and is distinct from the recruitment of PEN1 syntaxin in a plasma membrane microdomain beneath fungal appressoria (*6*, *7*). Independent biochemical fractionation of crude leaf protein extracts in soluble and microbody membrane fractions corroborated an association of wild-type PEN2 with microbody membrane fractions and revealed only trace amounts in enriched plasma membrane vesicles (fig. S3B). A substantial PEN2 portion was also found in corresponding soluble fractions, possibly due to a partial dissociation

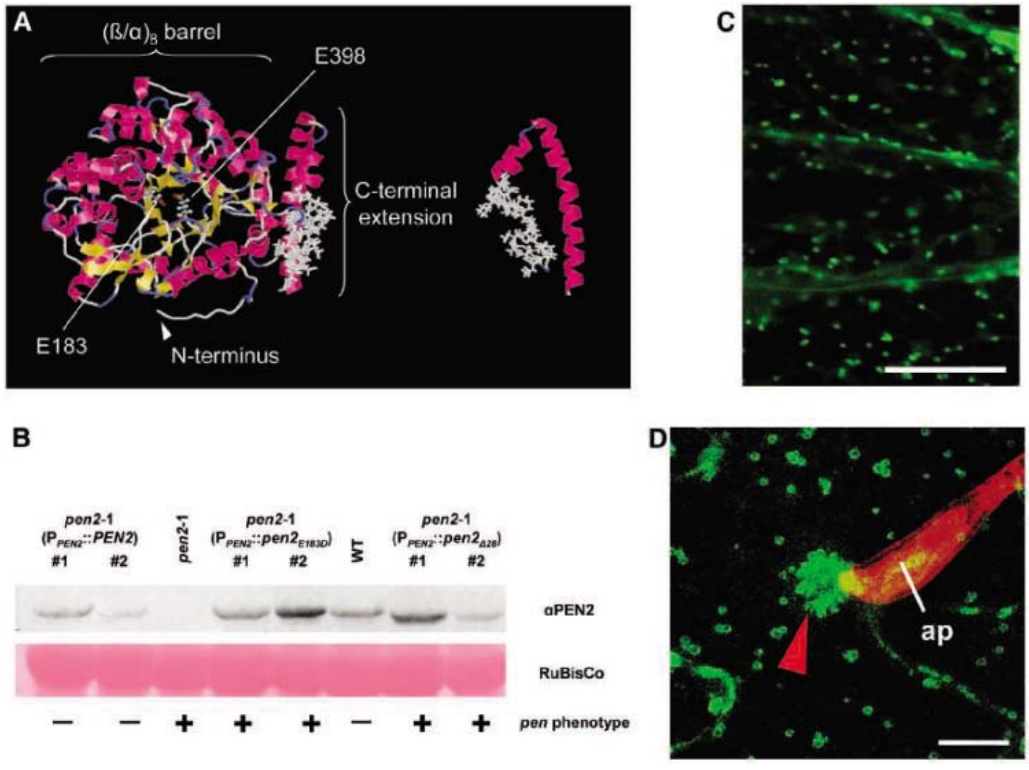


Fig. 2. Analysis of PEN2 function and subcellular localization. (**A**) Topview ribbon diagram of a PEN2 structure model. The catalytic machinery consists of two conserved acid/base and nucleophilic glutamates (E183 and E398). The unique C-terminal extension (90° rotated view shown separately on the right) includes a low complexity region (white ball and stick presentation) followed by a predicted helical region of 28 amino acids. (**B**) Immunodetection of PEN2 in crude leaf protein extracts using a PEN2 antibody. PEN2 accumulation in independent transgenic lines (labeled 1 and 2) expressing wild-type *PEN2* or catalytically inactive ($pen2_{E183D}$) or C-terminally truncated ($pen2_{\Delta 28}$) versions driven by native 5′ regulatory sequences (P_{PEN2}). Complementation of the *pen2* phenotype after *Bgh* inoculation experiments is denoted by + or – below each lane. Ponceau S detection of RuBisCo was used as protein loading control. (**C**) Confocal laser scanning microscopy of leaf epidermal cells in transgenic *pen2*-1 mutants expressing a functional PEN2-GFP fusion reveals association with vesicle-like bodies. Scale bar, 10 μm. (**D**) Peroxisomes containing PEN2-GFP accumulate at *Bgh* entry sites (marked by red arrowhead). Fungal structures are stained in red color by propidium iodide. Scale bar, 10 μm. ap, appressorium.

from peroxisomal membranes during the fractionation procedure.

Although the identified *pen* single mutants (*pen1*, *pen2*, and *pen3*) or the double mutant *pen1 pen2* compromise nonhost resistance at the cell periphery, each mutant line retains the ability to mount effective postinvasion immunity, which is linked to a cell death response (Fig. 1 and fig. S1B). Sustained postentry growth of powdery mildews requires a haustorial complex in living epidermal cells to supply nutrients for hyphal growth on the leaf surface (epiphytic hyphae). The lipase-like EDS1 protein and its sequence-related interaction partners PAD4 and SAG101 are known to play a critical role in a subset of R protein–triggered and basal immune responses to invasive biotrophic pathogens (*13*). *Bgh* entry rates on single *eds1*, *pad4*, and *sag101* mutants were not significantly different from those found in wild type. Although interaction sites containing haustorial complexes showed an increased incidence of epiphytic fungal growth on each of these mutants relative to wild type, colonization attempts usually ceased after the formation of two to three elongating hyphae. However, we noted that about 2% of such sites on *pad4* and *eds1* plants permitted sustained epiphytic hyphal growth (i.e., microcolony formation) (Fig. 3A). Although the incidence of epiphytic fungal growth was further enhanced upon introgression of the *pen2* null mutation in the single mutants (forming *pen2 sag101*, *pen2 pad4*, and *pen2 eds1* double mutants), the frequency of microcolony formation remained low (Fig. 3A). Because the function of PAD4 and SAG101 in basal and R protein–triggered immunity were recently found to be partially redundant (*13*), we also challenged *pad4 sag101* double mutants with *Bgh*. Although pathogen entry rates on *pad4 sag101* lines were indistinguishable from that of wild type, microcolony formation was enhanced relative to those of the single mutants, suggesting similarly redundant activities of sequence-related PAD4 and SAG101 proteins in nonhost resistance (Fig. 3A). The incidence of microcolony formation was further increased in *pen2 pad4 sag101* triple mutants, revealing synergistic interactions between PEN2 and the redundant functions of PAD4 and SAG101 (Fig. 3A). We occasionally found conidiophores containing mature conidiospores on microcolonies of *pen2 pad4 sag101* leaves, indicating breakdown of nonhost resistance to the grass powdery mildew fungus (fig. S3D).

In interactions with *E. pisi*, entry rates of single *eds1*, *pad4*, and *sag101* mutants were not altered relative to those of wild-type plants, whereas epiphytic hyphal growth increased substantially on the former two mutant lines (fig. S3C). A synergistic effect was seen after introgression of *pen2* in *eds1* or *pad4* backgrounds (*pen2 eds1* and *pen2 pad4* plants), indicating separable functions for and synergistic activities of the respective wild-type genes in pre- and postinvasion resistance. Although sporulation was not detectable on the single mutants, both *pen2 eds1* and *pen2 pad4* plants supported *E. pisi* conidiospore formation (fig. S3C inserts). Similar to interactions with *Bgh*, SAG101 and PAD4 exerted redundant functions against *E. pisi* colonization, and impairment of these two postinvasion resistance components alone was sufficient to allow occasional *E. pisi* sporulation (fig. S3C). Absence of PEN2 in a *pad4 sag101* background (*pen2 pad4 sag101* triple mutants) greatly enhanced disease susceptibility such that the timing and extent of *E. pisi* colonization became macroscopically indistinguishable from wild-type *Arabidopsis* interactions with the virulent *G. orontii* species (Fig. 3B and fig. S3D). Collectively, these findings strongly suggest separate functions for PEN2 in preinvasion and the EDS1-PAD4-SAG101 signaling complex in postinvasion nonhost resistance to both nonadapted powdery mildews.

The pathogen-triggered accumulation of PEN2-containing peroxisomes reported in this study (fig. S3A) as well as the recruitment of PEN1 syntaxin in a plasma membrane microdomain at fungal entry sites (*6*, *7*) are inconsistent with a preformed and passive barrier against fungal ingress, implying instead the existence of an inducible resistance mechanism at the cell periphery. We have shown here conserved PEN1 and PEN2 functions in preinvasion resistance to both tested nonadapted powdery mildew species that, together, explain almost all failed entry attempts (*pen1 pen2* double mutants) (Fig. 1). A corollary of this finding is that host powdery mildews such as *G. orontii* must have evolved means to overcome or bypass PEN activities. Two lines of evidence suggest that PEN1 and

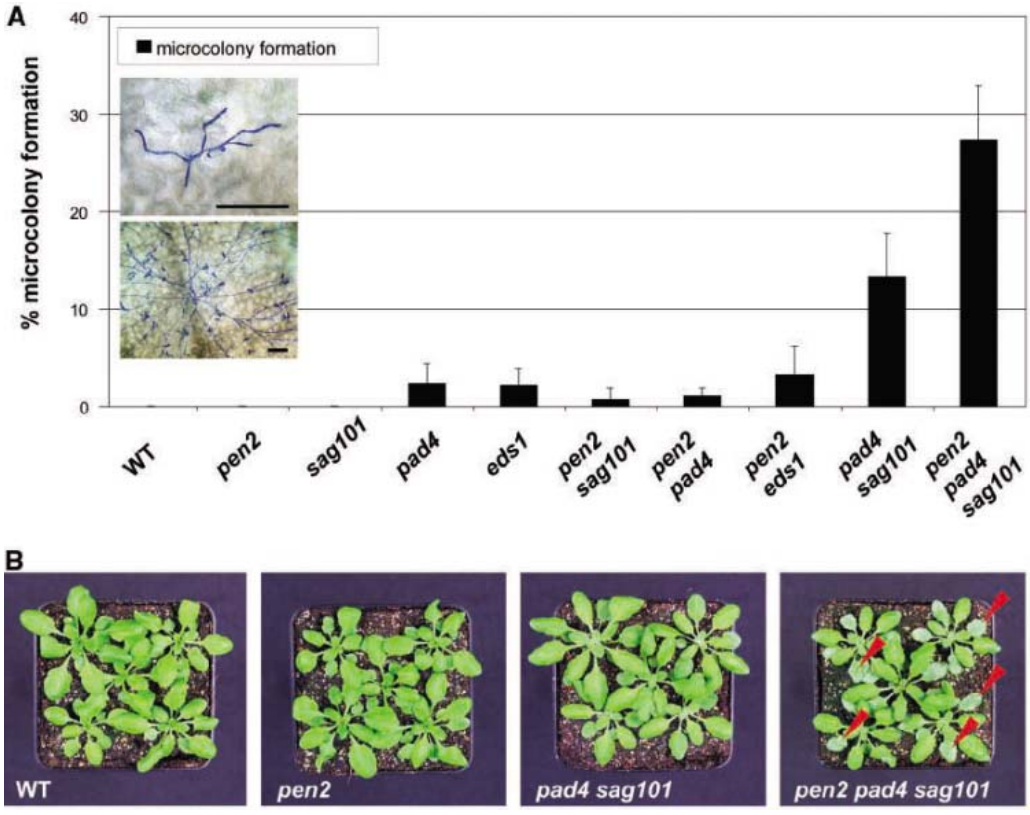


Fig. 3. Separable functions for PEN2 in preinvasion and the EDS1-PAD4-SAG101 signaling complex in postinvasion nonhost immunity. (**A**) Frequency of *Bgh* microcolonies on leaves of wild-type and mutant genotypes 7 days post–conidiospore inoculation (determined by calculating the incidence of microcolonies found per interaction site supporting epiphytic growth). The two light microscopic inserts show examples of a small (top) and a large (bottom) microcolony. The latter were only detectable on *pad4*-1 *sag101*-2 and *pen2*-1 *pad4*-1 *sag101*-2 lines. Error bars indicate standard deviations for triplicate measurements. (**B**) Macroscopic infection phenotypes of wild type and the indicated mutant genotypes 7 days post–*E. pisi* conidiospore inoculation. Red arrowheads denote leaves of *pen2*-1 *pad4*-1 *sag101*-2 plants that are densely covered by sporulating *E. pisi* fungal mycelium.

PEN2 represent components of two distinct entry resistance mechanisms: the broader spectrum of biological activity of PEN2 and the accumulative effect seen in *pen1 pen2* double null mutants in interactions with *Bgh*. Thus, we consider a simple model in which cargo of PEN2-containing peroxisomes is secreted via a putative PEN1 ternary SNARE complex unlikely. Failure of the $PEN2_{E183D}$ variant to complement the *pen2* mutant phenotype indicates that catalytic activity is required for PEN2 function. This makes it unlikely that PEN2 acts indirectly through interactions with other proteins. However, the peroxisomal localization and focal accumulation of PEN2 at fungal entry sites suggests that subcellular localization is an additional determinant for PEN2 function. The congregation of PEN2-containing peroxisomes at fungal entry sites might provide a mechanism for the activation and release of a small molecule at a high concentration. Because PEN2 inhibits *in planta* the pathogenesis of diverse fungal parasites, its catalytic product might directly or indirectly exert broad-spectrum toxic activity. Irrespective of this, our findings reveal a link between immune responses and a genetically defined peroxisomal component. Conceptually, the proposed pathogen-triggered focal secretory process, including PEN1 syntaxin and concentration of PEN2 F1GH at fungal entry sites, is reminiscent of the polar secretion machinery in cytotoxic T cells that is induced upon T cell receptor recognition, leading to targeted release of lytic proteins and killing of target cells (*14*).

Although previous reports demonstrated the involvement of lipase-like EDS1 in nonhost resistance to the grass powdery mildew fungus (*15*, *16*), we have shown here that the combined PAD4 and SAG101 contributions to postinvasion nonhost resistance greatly exceed those of the single components EDS1, PAD4, or SAG101. Our findings of redundant PAD4 and SAG101 activities in nonhost resistance are reminiscent of recently reported redundant PAD4 and SAG101 signaling functions in basal and R protein–triggered immunity (*13*), thereby pointing to the involvement of plant immune receptors in triggering nonhost resistance. One possible receptor class that could operate in nonhost resistance and act via PAD4 and SAG101 signaling would be the PAMP receptors recognizing conserved pathogen-associated molecular patterns (*17*). Consistent with this, flagellin perception by the FLS2 receptor-like kinase leads to rapid transcriptional activation of *EDS1*, *PAD4*, and *SAG101* as well as *PEN1*, *PEN2*, and *PEN3* (*18*). Thus, it is possible that pre- and postinvasion nonhost resistance components represent two branch pathways activated by the same immune receptors or are components of two pathways that are sequentially activated by different receptors. One potential advantage of the inferred two-layer concept for nonhost resistance to powdery mildews is robustness through functional redundancy, because pre- and postinvasive immune responses are each sufficient to terminate fungal pathogenesis.

References and Notes

1. J. Mengaud, H. Ohayon, P. Gounon, R. M. Mege, P. Cossart, *Cell* **84**, 923 (1996).
2. R. Panstruga, P. Schulze-Lefert, *Microbes Infect.* **5**, 429 (2003).
3. L. E. B. Johnson, W. R. Bushnell, R. J. Zeyen, *Can. J. Bot.* **60**, 1068 (1982).
4. D. G. Mellersh, I. V. Foulds, V. J. Higgins, M. C. Heath, *Plant J.* **29**, 257 (2002).
5. N. C. Collins *et al.*, *Nature* **425**, 973 (2003).
6. F. F. Assaad *et al.*, *Mol. Biol. Cell* **15**, 5118 (2004).
7. R. A. Bhat, M. Miklis, E. Schmelzer, P. Schulze-Lefert, R. Panstruga, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3135 (2005).
8. J. S. Bonifacino, B. S. Glick, *Cell* **116**, 153 (2004).
9. G. S. Saenz, J. W. Taylor, *Can. J. Bot.* **77**, 150 (1999).
10. Z. Xu *et al.*, *Plant Mol. Biol.* **55**, 343 (2004).
11. M. Czjzek *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13555 (2000).
12. K. Nakai, P. Horton, *Trends Biochem. Sci.* **24**, 34 (1999).
13. B. J. Feys *et al.*, *Plant Cell* **17**, 2601 (2005).
14. G. Bossi, G. M. Griffiths, *Semin. Immunol.* **17**, 87 (2005).
15. B. W. Yun *et al.*, *Plant J.* **34**, 768 (2003).
16. L. Zimmerli, M. Stein, V. Lipka, P. Schulze-Lefert, S. Somerville, *Plant J.* **40**, 633 (2004).
17. C. Zipfel, G. Felix, *Curr. Opin. Plant Biol.* **8**, 353 (2005).
18. C. Zipfel *et al.*, *Nature* **428**, 764 (2004).

19. We thank H. Häweker and B. Pickel for excellent technical assistance, B. Weisshaar for providing *PEN2* T-DNA insertion line *pen2*-2 (GABI-KAT 134C04), and I. Somssich for critical reading of the manuscript. This work was supported by the GABI-NONHOST consortium funded by Bundesministerium für Bildung und Forschung and BASF Plant Science. A.M. was funded by the Ministerio de Educacion y Ciencia, Spain (grant BIO2003-4424).

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1180/DC1
Materials and Methods
Figs. S1 to S3

26 August 2005; accepted 18 October 2005
10.1126/science.1119409

GTF2IRD1 in Craniofacial Development of Humans and Mice

May Tassabehji,[1]* Peter Hammond,[2] Annette Karmiloff-Smith,[3] Pamela Thompson,[1] Snorri S. Thorgeirsson,[4] Marian E. Durkin,[4] Nicholas C. Popescu,[4] Timothy Hutton,[2] Kay Metcalfe,[1] Agnes Rucka,[1] Helen Stewart,[5] Andrew P. Read,[1] Mark Maconochie,[6] Dian Donnai[1]

Craniofacial abnormalities account for about one-third of all human congenital defects, but our understanding of the genetic mechanisms governing craniofacial development is incomplete. We show that *GTF2IRD1* is a genetic determinant of mammalian craniofacial and cognitive development, and we implicate another member of the TFII-I transcription factor family, *GTF2I*, in both aspects. *Gtf2ird1*-null mice exhibit phenotypic abnormalities reminiscent of the human microdeletion disorder Williams-Beuren syndrome (WBS); craniofacial imaging reveals abnormalities in both skull and jaws that may arise through misregulation of *goosecoid*, a downstream target of *Gtf2ird1*. In humans, a rare WBS individual with an atypical deletion, including *GTF2IRD1*, shows facial dysmorphism and cognitive deficits that differ from those of classic WBS cases. We propose a mechanism of cumulative dosage effects of duplicated and diverged genes applicable to other human chromosomal disorders.

The vertebrate head is a complex structure whose assembly is coordinated by a hierarchy of regulatory genes, many of which are members of transcription factor families (e.g., *Hox*, *Msx*, *Dlx*) (*1*). The *TFII-I* gene family encodes related transcription factors that all contain a leucine zipper and multiple I-repeat motifs, whose complex and incompletely defined biology includes regulation of genes important in vertebrate development (*2*–*4*). The three family members are *GTF2I* (or *TFII-I*) (*5*), *GTF2IRD1* (or *GTF3*, *MusTRD1*, *BEN*, *WBSCR11*) (*6*, *7*), and *GTF2IRD2* (*8*). *GTF2IRD1* and *GTF2I* are invariably deleted in WBS (*9*) as part of a larger deletion. GTF2I, the first member identified, acts as a basal transcription factor that binds to initiator elements of various promoters and also regulates transcription through E-box elements at enhancers in response to upstream signaling events (*5*). GTF2IRD1 can bind regulatory elements upstream of genes involved in tissue development and differentiation (*3*, *4*). In *Xenopus*, GTF2IRD1 (or XWBSCR11) acts as a positive regulator of *goosecoid* in response to the transforming growth factor–β family member activin (*3*); during mouse embryonic development, Gtf2ird1 interacts with the enhancer that controls early-phase *Hoxc8* expression (*4*). In humans, heterozygous deletions on chromosome 7q11.23, including these genes, are the genetic basis of the multisystem developmental disorder WBS (*9*).

Individuals with WBS have characteristic dysmorphic facies alongside other developmental abnormalities, including vascular problems (especially supravalvular aortic stenosis, SVAS), short stature, and a unique cognitive profile (WBSCP), with relatively proficient language and face-processing skills but serious impairments in spatial and numerical ability (*10*, *11*). Personality traits include overfriendliness and charismatic speech. Many experience anxiety and simple phobias. The roots of the mental and cognitive aspects probably lie in disruption of normal neurodevelopment, because brain morphology and neural organization are abnormal (*12*). At the molecular level, WBS is a contiguous gene disorder that involves a heterozygous deletion of ~1.5 megabase (Mb) encompassing some 28 genes (*13*, *14*) (Fig. 1A). The only firm genotype-phenotype correlation is between vascular problems caused by haploinsufficiency for the elastin gene (*9*).

Attempts to identify the genes underlying the craniofacial, cognitive, and behavioral features of WBS rely on the identification of rare individuals with smaller deletions in the WBS region, supplemented by studies of mouse models. Early links made between haploinsufficiency for *LIMK1* and the characteristic cognitive profile (*15*) are ambiguous and are contradicted by reports of individuals who have this gene deleted but lack the WBSCP (*16*). An SVAS patient (*16*), CS, with 23 of the 28 defined genes in the critical region deleted (including *LIMK1*) (Fig. 1A), has none of the craniofacial, cognitive, or behavioral abnormalities that are seen in individuals with the full deletion. CS retains genes at the telomeric end of the critical region, which suggests that the decisive genes responsible for craniofacial and neurological abnormalities are located in this region (which harbors the *TFII-I* gene family), a claim supported by other patient studies (*16*–*18*). Here, we show that both *GTF2IRD1* and *GTF2I* are responsible for the main aspects of WBS.

We have identified an atypical WBS individual, HR, with a smaller genetic deletion relative to classic WBS cases but a larger deletion than in SVAS patient CS, including two extra telomeric genes, *CYLN2* and *GTF2IRD1*. HR is a 4.5-year-old girl with surgically corrected pulmonary artery stenosis. Her birthweight (3380 g) and growth appear normal, and at 4.5 years her height is just above the 50th centile. Her facial features are suggestive of, but not classical for, WBS (Fig. 2A). Early developmental milestones such as sitting and walking were within normal limits. However, at 18 months (and unlike her older sibling at the same age) she had a vocabulary of only a few single words, and by age 4 she continued to show a delay in language acquisition as well as serious deficits in spatial cognition [not to the degree seen in WBS (*19*)]. She does not exhibit the overly friendly personality.

The WBS-like dysmorphic features of HR were quantified using three-dimensional (3D) face surface images captured with stereo photogrammetric devices. Images were compared unseen to dense surface models (DSMs) of face shape constructed from a collection of 185 control and 85 WBS individuals aged 2 weeks to 20 years (Fig. 2B). Such comparisons can reliably distinguish between dysmorphic and normal faces (92% discriminating accuracy) (*20*, *21*). HR is on the periphery of both the WBS and control groups and is classified as having mild, but not classic, WBS features (Fig. 2C). Because some members (adults and children) of two families with a smaller deletion (*ELN* + *LIMK1*) have been reported with some dysmorphic features (*15*), we compared 3D face images captured from two adults with a similar deletion (PM and TM, Fig. 1A), but no phenotype other than SVAS, to DSMs constructed from 132 control and 45 WBS adults. No WBS-like features were present (fig. S1). Their childhood photographs also showed no facial dysmorphology.

Chromosome analysis of HR with an *ELN*/*LIMK1* probe identified a heterozygous deletion at 7q11.23. The extent of the deletion was determined by polymerase chain reaction (PCR) analysis of DNA isolated from somatic cell hybrids containing either the deleted or the normal copy of chromosome 7. These data indicate that the heterozygous deletion in HR spans ~1 Mb encompassing the interval between genes *NOL1R* and *GTF2IRD1* (Fig. 1A) (fig. S2). The proximal breakpoint lies within

[1]Academic Unit of Medical Genetics, University of Manchester, St. Mary's Hospital, Manchester M13 9PL, UK. [2]Eastman Dental Institute, [3]Institute of Child Health, University College London, London WC1E 6BT, UK. [4]National Cancer Institute, Bethesda, MD 20892, USA. [5]Churchill Hospital, Oxford OX3 7LJ, UK. [6]School of Life Sciences, University of Sussex, Brighton BN1 9RH, UK.

*To whom correspondence should be addressed. E-mail: m.tassabehji@manchester.ac.uk

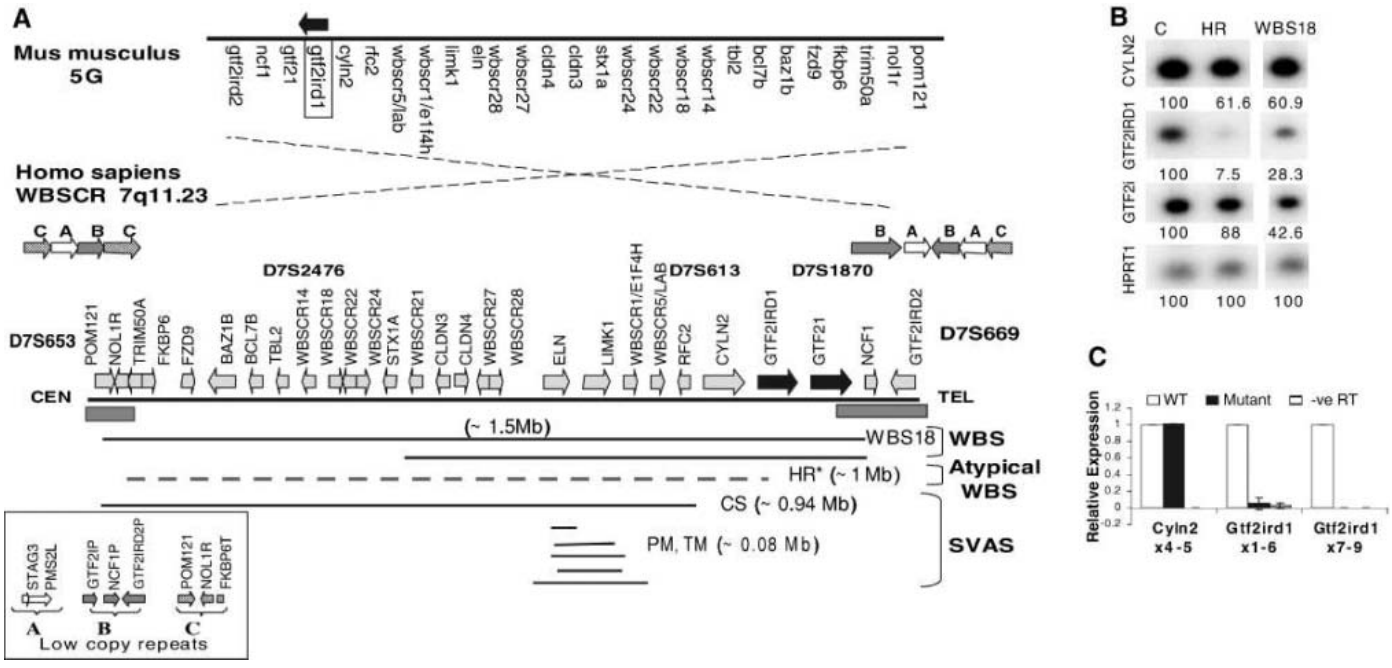
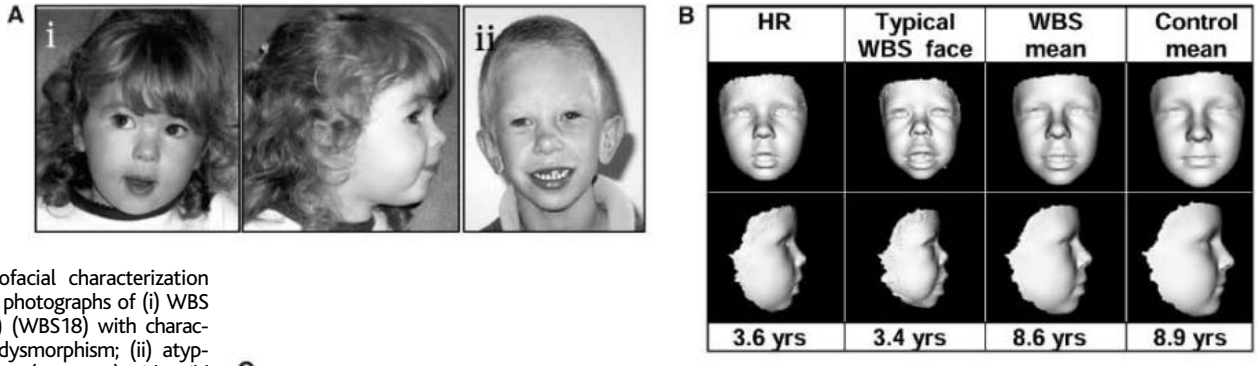


Fig. 1. (**A**) Transcript map of the WBS region on human chromosome 7q11.23 showing key individuals with partial deletions (*15*–*18*). WBS18 has classic WBS (~1.5 Mb deletion); HR has atypical WBS (~1 Mb deletion); CS has SVAS (~0.94 Mb deletion). Gray boxes denote areas where WBS deletion breakpoints cluster. CEN, centromere; TEL, telomere; HR, CS, PM, and TM are UK patients. The syntenic mouse region on chromosome 5G1 retains gene order but is inverted (not to scale). (**B**) Gene expression analysis in HR by RT-PCR. Left to right: control, HR, and WBS18. Numbers represent percentage of gene expression relative to the normal individual and normalized against the *HPRT1* control. HR and WBS18 show reduced *CYLN2* and *GTF2IRD1* expression. (**C**) Gene expression analysis in mice by quantitative real-time RT-PCR. The histogram shows the relative quantities of *Gtf2ird1* normalized against the control gene *Lamc1* in adult wild-type (WT) and mutant (*Gtf2ird1*-null) mice (-ve RT, negative RT blank control). *Gtf2ird1* expression is absent in the mutant mice.



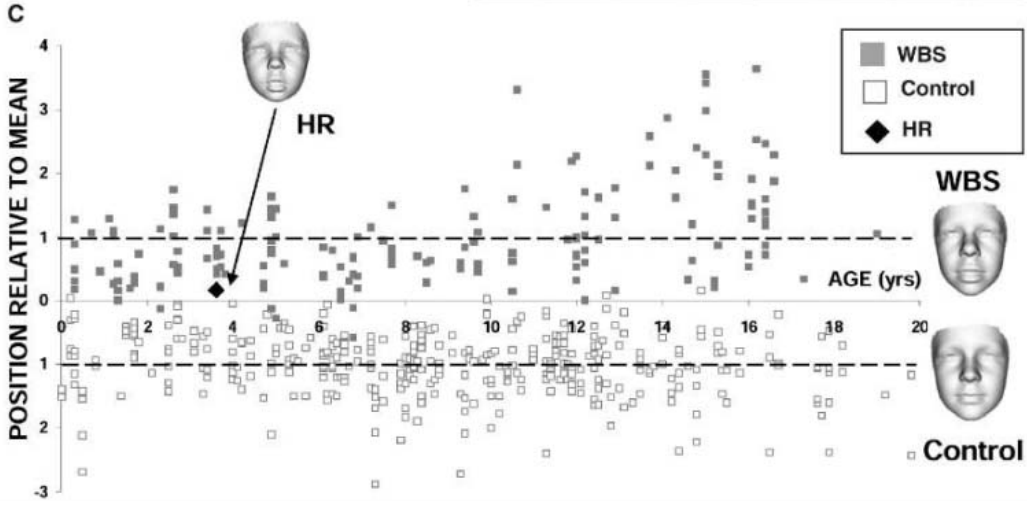


Fig. 2. Craniofacial characterization of HR. (**A**) 2D photographs of (i) WBS child (4 years) (WBS18) with characteristic facial dysmorphism; (ii) atypical individual HR (3.5 years) with mild dysmorphic features. Note periorbital fullness, long philtrum, upturned nose. (**B**) 3D face surfaces of HR and a typical female WBS child alongside averages of control and WBS subgroups. Each is synthesized in a DSM made up of all 270 images (excluding HR's). (**C**) Scatterplot display of the unseen classification of HR against the control and WBS DSMs. Horizontal broken lines represent the position of the mean control and WBS faces at –1 and +1, respectively. Vertical axis: position of each face relative to the means normalized to [–1,+1]; horizontal axis: age in years. Background scatterplot represents the classification of the unseen test sets used to determine the average discrimination performance of the 20 DSMs built with the randomly generated training sets. Overlaid is the average position of the unseen closest mean classification of HR's image estimated using the 20 DSMs. HR is classified as borderline (i.e., mildly dysmorphic).

the centromeric low-copy repeat (C-mid), comprising pseudogenes, repeats, and partial genes (*14*). The distal breakpoint is located ~7 kb downstream of exon 1 and within intron 1 of *GTF2IRD1*. Intron 1 is large (53,895 base pairs) and rich in repeats (~53% content of short and long interspersed nuclear elements and long terminal repeats) that appear to make the region unstable and prone to microdeletions. Semiquantitative reverse transcription PCR (RT-PCR) of lymphoblastoid cell line RNA from HR revealed reduced expression of *GTF2IRD1* and normal levels of *GTF2I* expression (Fig. 1B). HR is therefore haploinsufficient for *GTF2IRD1*, even though the gene is only partially deleted and the translation start codon in exon 2 is still present.

To further study the effects of lack of GTF2IRD1, we used the transgenic mouse strain Tg(Alb1-Myc)166.8 (*22*) in which integration of a c-*myc* transgene on distal chromosome 5 has induced a deletion of ~40 kb in the mouse WBS syntenic region. The deletion starts downstream of *Cyln2* and includes the upstream transcription start site and exon 1 of *Gtf2ird1* while retaining the translation start site in exon 2. It therefore closely resembles the *GTF2IRD1* deletion breakpoint in HR. Gtf2ird1 expression was abolished in the transgenic line, whereas expression of *Cyln2* was unaffected (Fig. 1C) (fig. S3). These mice are prone to liver-specific hepatocellular carcinomas due to overexpression of the c-*myc* transgene (*22*), but our analysis has revealed additional phenotypes relevant to WBS.

In mice, loss of *Gtf2ird1* leads to growth retardation and craniofacial abnormalities. *Gtf2ird1*-null mutants are viable and fertile but smaller and lighter than wild-type controls (Fig. 3A). All *Gtf2ird1*-null mice display a characteristic facial appearance that includes periorbital fullness and a short snout (Fig. 3, B and C). About 20% of the homozygous mutants have a more severe craniofacial abnormality involving a misaligned jaw, which leads to chronic overgrowth of teeth, and a twisted snout (Fig. 3, B and C). This does not appear to affect neonatal suckling; however, once weaned, these mutants are unable to manipulate hard feed pellets. With a modified diet of soft mash and regular teeth clipping, their life-span is normal but they are smaller than their homozygous littermates from birth to adulthood (Fig. 3A).

Quantitative 3D facial scans of seven *Gtf2ird1*-null mice with the more severe phenotype were compared with scans from 10 wild-type mice. The craniofacial abnormality consistently involves both skull and jaws, in particular the periorbital area. *Gtf2ird1* mutants have a shorter snout than wild-type controls, and the face shape is typically asymmetric, with the snout twisted to the left or right by 7° to 12° (Fig. 3D) (fig. S4). Like *Gtf2ird1* mutant mice, WBS individuals also exhibit differing degrees of severity in their craniofacial dysmorphology, no doubt due to the influence of genetic background and modifier genes. *Gtf2ird1*-null mice also display a distinctive hind leg clasping and "kicking" reflex not seen in wild-type controls and suggestive of neurological dysfunction. Growth and craniofacial development appear normal in heterozygous $Gtf2ird1^{+/-}$ mice, which suggests that humans are more sensitive to the craniofacial effects (although apparently not the growth effects) of *GTF2IRD1* gene dosage.

Our findings show that homozygous loss of *Gtf2ird1* in mice results in craniofacial abnormalities reminiscent of those seen in WBS,

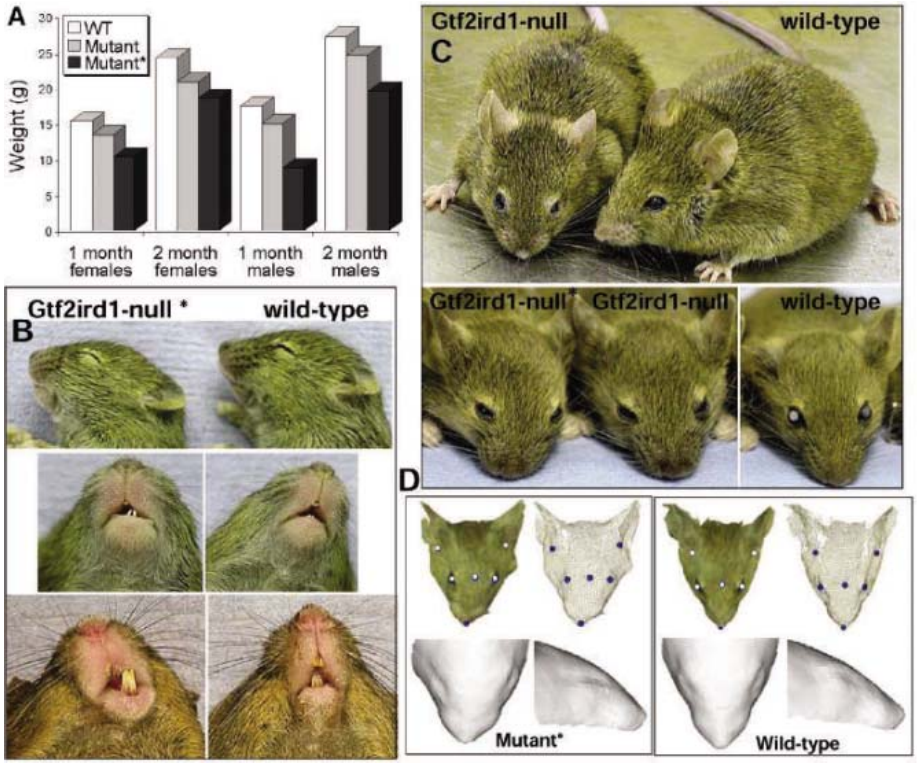


Fig. 3. (**A**) Growth deficiency in *Gtf2ird1*-null mice. Histogram showing body weights of wild-type (males, n = 13 to 24; females, n = 8), *Gtf2ird1*-null (males, n = 26 to 27; females, n = 10 to 14), and *Gtf2ird1*-null mice with misaligned jaws (Mutant*) (males, n = 8; females, n = 9) at ages 1 and 2 months. All mutants have a lower body weight relative to the wild type (9.4 to 13.9% decrease, depending on age); mutants with misaligned jaws (*Gtf2ird1*-null*) have the lowest body weights (24 to 42% decrease, depending on age). Pairwise comparisons show that differences in body weight between the genotypes are statistically significant. (Two-tailed t tests; P values of wild-type relative to mutant mice at 41 and 2 months, respectively: females, 4.618×10^{-3}, 3.72×10^{-4}; males, 2.79549×10^{-6}, 3.64363×10^{-5}. P values of wild-type relative to mutant* mice at 41 and 2 months, respectively: females, 1.25987×10^{-7}, 1.15×10^{-4}; males, 6.41166×10^{-14}, 1.62929×10^{-7}.) (**B**) Craniofacial abnormalities. Misaligned jaw and shorter snout of *Gtf2ird1*-null mice at ages 12 days and 9 months (top and bottom panels, respectively). (**C**) Adult *Gtf2ird1*-null mice display a smaller body size, a dysmorphic face with "periorbital fullness," and a shorter snout. Lower left panel shows a *Gtf2ird1*-null mutant (*) with a twisted snout. (**D**) 2D images of 3D surface scans of two age-matched mice comparing face shape. Top panels show a wild-type and a *Gtf2ird1*-null male with a twisted snout (mutant*). Bottom panels show the average 3D surfaces of wild-type (n = 10) and mutant* (n = 7) mice.

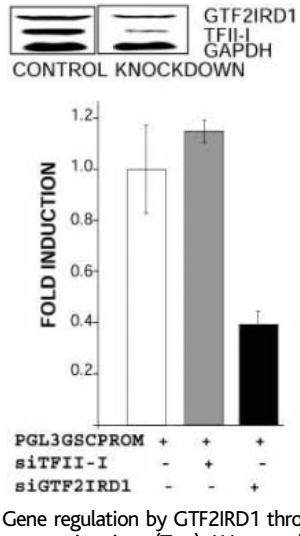


Fig. 4. Gene regulation by GTF2IRD1 through the *GSC* promoter in vivo. (**Top**) Western blots of siRNA-transfected HEK-293T cell lysates. Both endogenous *GTF2IRD1* and *TFII-I* are knocked down. (**Bottom**) RNA interference assays. HEK-293T cells were transfected with a *GSC* promoter–luciferase gene reporter construct (PGL3-*GSC*prom) and with either *GTF2IRD1* or *TFII-I* siRNAs. Knockdown of GTF2IRD1 induced ~60% down-regulation of luciferase activity (mean ± SEM of triplicates). *TFII-I* control siRNA has no effect under these conditions.

together with growth retardation and neurological abnormalities. This is consistent with the expression pattern of *Gtf2ird1* in the developing brain and craniofacial areas (*23*) and its ability to regulate expression of *goosecoid* (*Gsc*) and *Hoxc8*, genes that control craniofacial and skeletal development (*3*, *4*). *Gsc*-null mice die soon after birth with craniofacial defects, rib fusions, and sternum abnormalities (*24*); *Hoxc8* $EE^{-/-}$ mice, where temporal regulation of *Hoxc8* is altered but not abolished, have skeletal pathologies and signs of neurological dysfunction (*25*). We used a short interfering RNA (siRNA) that knocks down levels of endogenous GTF2IRD1 by ~60% to show regulation of *GSC* by GTF2IRD1 in human embryonic kidney (HEK) 293T cells. In cotransfection experiments, the GTF2IRD1 siRNA reduced expression of a pGL3-*GSC*prom luciferase reporter construct (Fig. 4), suggesting that GTF2IRD1 influences gene transcription at endogenous levels. To date, few upstream regulators of *Gsc* have been defined in vivo, even though its developmental importance in *Drosophila* and humans is well established. Misregulation of *GSC* expression due to absence or lower levels of GTF2IRD1 probably contributes to the craniofacial pathologies seen in WBS and *Gtf2ird1*-null mice.

Of the other genes deleted in HR, *CYLN2* probably contributes to, but is not solely responsible for, the neurological phenotype in WBS. Although no human case of isolated haploinsufficiency for *CYLN2* has been reported, $Cyln2^{-/-}$ and $Cyln2^{+/-}$ mice present with mild structural brain abnormalities, hippocampal dysfunction, and deficits in motor coordination, alongside mild growth deficiency but no craniofacial defects (*26*). However, haploinsufficiency for *CYLN2* and *GTF2IRD1* alone cannot explain all the clinical features of WBS, because HR displays a milder clinical profile. The more pronounced facial and cognitive/behavioral phenotypes associated with the larger deletion implicate other telomeric genes in these features, specifically *GTF2I*, which lies distal to *GTF2IRD1* and is deleted in classic WBS cases. GTF2I and GTF2IRD1 share many structural properties and are likely to have overlapping functions; indeed, both have been shown to regulate gene activity through a common DNA element, DICE (*27*). This work introduces members of the *TFII-I* gene family as critical regulators of craniofacial and neurological development.

Our findings allow us to compile a picture of the molecular pathology of WBS, a classical human microdeletion syndrome. No single gene is responsible for the craniofacial or cognitive features of WBS. We suggest that cumulative dosage of *TFII-I*–family genes explains the main phenotypes. *Gtf2ird1*-null mice and classic WBS individuals have two functioning copies (in trans and cis, respectively), whereas HR has three functioning genes of the *GTF2IRD1*/*GTF2I* cluster and shows milder WBS phenotypes. Whether one gene has more effect than the other or whether the effects are additive or multiplicative remains to be determined. Haploinsufficiency for other genes in the WBS critical region explains the vascular features and may contribute to the full syndrome, but it is not the main cause. Because adjacent duplicated but diverged genes are common in the human genome, such cumulative dosage effects may underlie the pathology of other chromosomal syndromes where members of different gene families are deleted or duplicated.

References and Notes

1. S. Alappat, Z. Y. Zhang, Y. P. Chen, *Cell Res.* **13**, 429 (2003).
2. T. A. Hinsley, P. Cunliffe, H. J. Tipney, A. Brass, M. Tassabehji, *Protein Sci.* **13**, 2588 (2004).
3. C. Ring *et al.*, *Genes Dev.* **16**, 820 (2002).
4. D. Bayarsaihan, F. H. Ruddle, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7342 (2000).
5. A. L. Roy *et al.*, *EMBO J.* **16**, 7091 (1997).
6. M. Tassabehji *et al.*, *Eur. J. Hum. Genet.* **7**, 737 (1999).
7. J. V. O'Mahoney *et al.*, *Mol. Cell. Biol.* **18**, 6641 (1998).
8. H. J. Tipney, T. A. Hinsley, A. Brass, D. Donnai, M. Tassabehji, *Eur. J. Hum. Genet.* **12**, 551 (2004).
9. A. K. Ewart *et al.*, *Nat. Genet.* **5**, 11 (1993).
10. C. A. Morris, S. A. Demsey, C. O. Leonard, C. Dilts, B. L. Blackburn, *J. Pediatr.* **113**, 318 (1988).
11. D. Donnai, A. Karmiloff-Smith, *Am. J. Med. Genet.* **97**, 164 (2000).
12. A. Reiss, S. Eliez, S. J. E. Schmitt, *J. Cogn. Neurosci.* **12** (suppl.), 65 (2000).
13. M. Tassabehji, *Hum. Mol. Genet.* **15**, 229 (2003).
14. M. Bayes, L. F. Magano, N. Rivera, R. Flores, L. A. Perez Jurado, *Am. J. Hum. Genet.* **73**, 131 (2003).
15. J. M. Frangiskakis *et al.*, *Cell* **86**, 59 (1996).
16. M. Tassabehji *et al.*, *Am. J. Hum. Genet.* **64**, 118 (1999).
17. A. Botta *et al.*, *J. Med. Genet.* **36**, 478 (1999).
18. R. Heller, A. Rauch, S. Luttgen, B. Schroder, A. Winterpacht, *J. Med. Genet.* **40**, E99 (2003).
19. A. Karmiloff-Smith *et al.*, data not shown.
20. P. Hammond *et al.*, *Am. J. Med. Genet. A* **126**, 339 (2004).
21. P. Hammond *et al.*, *Am. J. Hum. Genet.*, in press.
22. M. E. Durkin, C. L. Keck-Waggoner, N. C. Popescu, S. S. Thorgeirsson, *Genomics* **73**, 20 (2001).
23. J. A. Rivera-Pérez, M. Mallo, M. Gendron-Maguire, T. Gridley, R. R. Behringer, *Development* **121**, 3005 (1995).
24. A. H. Juan, F. H. Ruddle, *Development* **130**, 4823 (2003).
25. D. Bayarsaihan *et al.*, *Gene Expr. Patterns* **3**, 579 (2003).
26. C. C. Hoogenraad *et al.*, *Nat. Genet.* **32**, 116 (2002).
27. D. Tantin, M. I. Tussie-Luna, A. L. Roy, P. A. Sharp, *J. Biol. Chem.* **279**, 5460 (2004).

28. Supported by Wellcome Trust grant 061183 (M.T.). Scanners were funded by BDF NewLife (P.H.). Surfim loaned specialist scanning equipment. We thank the families and W. Fergusson, M. J. Carrette, and W. Beckett for their assistance.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1116142/DC1
Materials and Methods
Figs. S1 to S4
References

15 June 2005; accepted 5 October 2005
Published online 3 November 2005;
10.1126/science.1116142
Include this information when citing this paper.

DISC1 and PDE4B Are Interacting Genetic Factors in Schizophrenia That Regulate cAMP Signaling

J. Kirsty Millar,[1]*† Benjamin S. Pickard,[1]* Shaun Mackie,[1] Rachel James,[1] Sheila Christie,[1] Sebastienne R. Buchanan,[1] M. Pat Malloy,[1] Jennifer E. Chubb,[1] Elaine Huston,[2] George S. Baillie,[2] Pippa A. Thomson,[1] Elaine V. Hill,[2] Nicholas J. Brandon,[3] Jean-Christophe Rain,[4] L. Miguel Camargo,[3] Paul J. Whiting,[3] Miles D. Houslay,[2] Douglas H. R. Blackwood,[1,5] Walter J. Muir,[1,5] David J. Porteous[1]

The *disrupted in schizophrenia 1* (*DISC1*) gene is a candidate susceptibility factor for schizophrenia, but its mechanistic role in the disorder is unknown. Here we report that the gene encoding phosphodiesterase 4B (*PDE4B*) is disrupted by a balanced translocation in a subject diagnosed with schizophrenia and a relative with chronic psychiatric illness. The PDEs inactivate adenosine 3′,5′-monophosphate (cAMP), a second messenger implicated in learning, memory, and mood. We show that DISC1 interacts with the UCR2 domain of PDE4B and that elevation of cellular cAMP leads to dissociation of PDE4B from DISC1 and an increase in PDE4B activity. We propose a mechanistic model whereby DISC1 sequesters PDE4B in resting cells and releases it in an activated state in response to elevated cAMP.

Schizophrenia and bipolar affective disorder are common, debilitating conditions that are determined in part by genetic factors. The *DISC1* gene (for *disrupted in schizophrenia 1*) is a candidate susceptibility factor for psychiatric illness with both genetic and biological plausibility (*1*). *DISC1* is disrupted by a balanced chromosomal translocation t(1;11)(q42;q14) cosegregating with schizophrenia (7 cases), bipolar affective disorder (1 case), and related affective disorders (10 cases) in a large Scottish family [maximum logarithm of the odds ratio for linkage (lod score) = 7.1] (*2*, *3*). Translocation carriers, even without a major

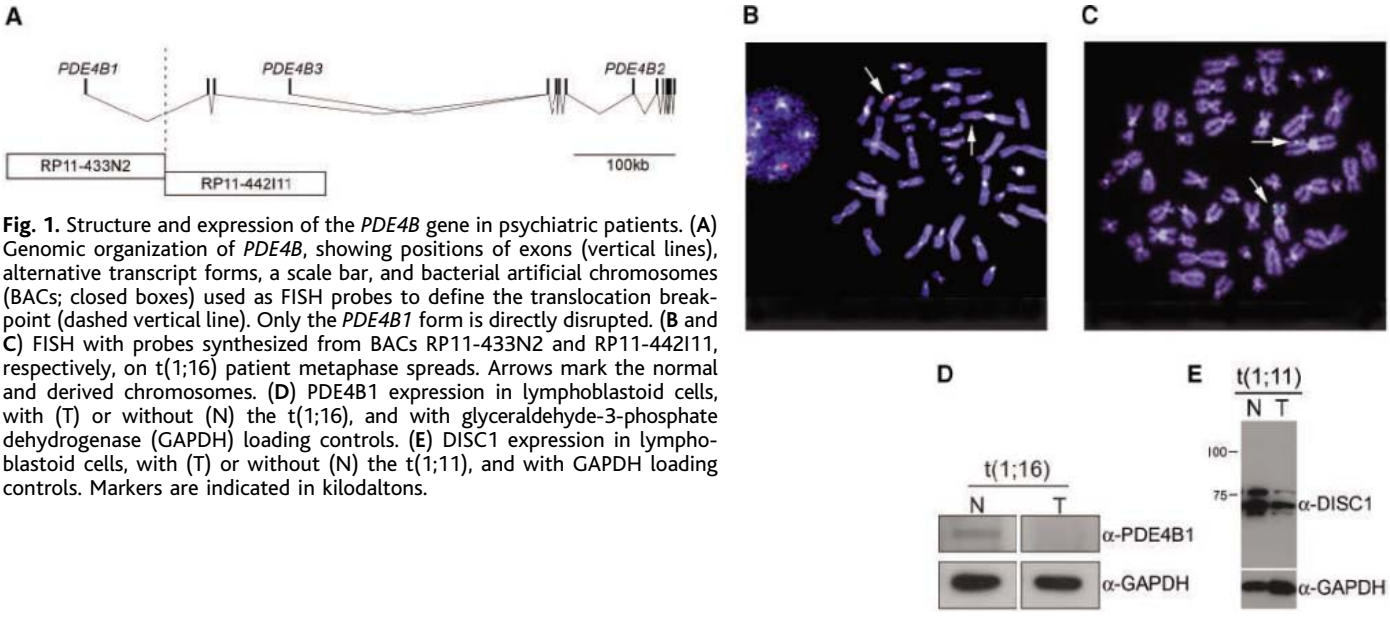


Fig. 1. Structure and expression of the *PDE4B* gene in psychiatric patients. (**A**) Genomic organization of *PDE4B*, showing positions of exons (vertical lines), alternative transcript forms, a scale bar, and bacterial artificial chromosomes (BACs; closed boxes) used as FISH probes to define the translocation breakpoint (dashed vertical line). Only the *PDE4B1* form is directly disrupted. (**B** and **C**) FISH with probes synthesized from BACs RP11-433N2 and RP11-442I11, respectively, on t(1;16) patient metaphase spreads. Arrows mark the normal and derived chromosomes. (**D**) PDE4B1 expression in lymphoblastoid cells, with (T) or without (N) the t(1;16), and with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading controls. (**E**) DISC1 expression in lymphoblastoid cells, with (T) or without (N) the t(1;11), and with GAPDH loading controls. Markers are indicated in kilodaltons.

psychiatric diagnosis, have reduced amplitudes of the P300 event-related potential, consistent with an underlying cognitive defect (*3*). A number of independent genetic linkage and association studies provide confirmatory evidence for involvement of the *DISC1* locus in schizophrenia, schizoaffective disorder, and bipolar affective disorder (*1*–*4*). DISC1 is expressed in regions of the brain implicated in the genesis of psychiatric symptoms (*5*–*7*) and binds diverse proteins in the central nervous system, including the neurodevelopmental proteins FEZ1 (*8*) and NUDEL (*9*, *10*).

In ongoing studies of psychiatric patients with chromosomal abnormalities, we identified a proband with schizophrenia who carried a balanced t(1;16)(p31.2;q21) translocation. The subject had a history of repeated psychotic episodes with auditory hallucinations and delusions and was treated with standard antipsychotic medications. A cousin of the proband who also carried the translocation had a psychotic illness with prolonged hospital admission but was not available for interview (*11*). Using fluorescence in situ hybridization (FISH) (*11*), we showed that the translocation breakpoint on chromosome 16 lies within an intron of the

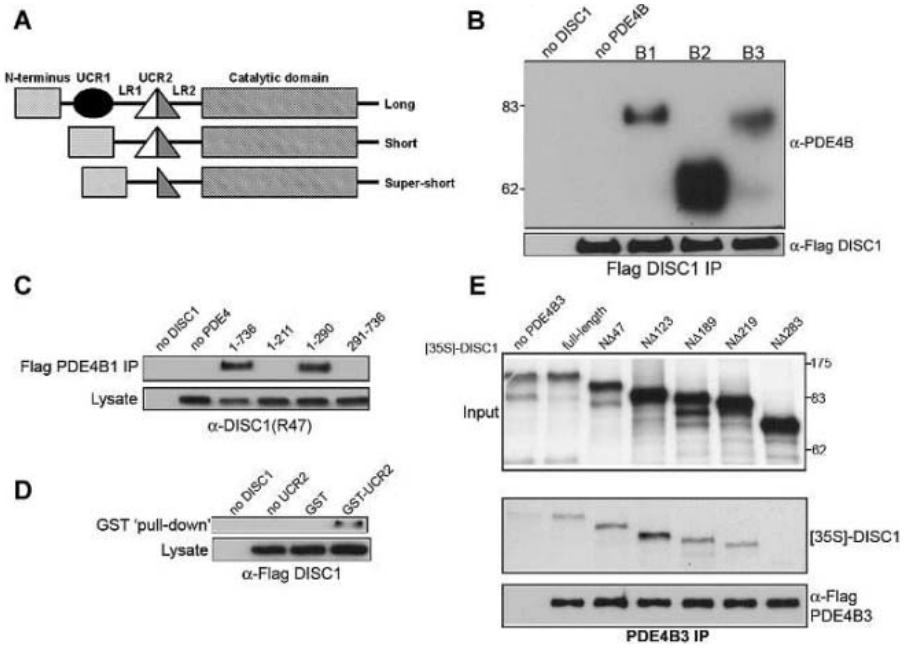


Fig. 2. Human DISC1 binds the PDE4B UCR2 domain in transfected cells. (**A**) Domain organization of type 4 phosphodiesterases. The common structure of core regions forming the UCR1/UCR2 module and the catalytic domain are indicated. Each PDE4 isoform contains a unique N terminus. (**B**) HEK293 cells were cotransfected with DISC1 and PDE4 expression vectors. FLAG epitope–tagged immunoprecipitates from lysates prepared from HEK293 cells cotransfected with either empty vector or FLAG-DISC1 and PDE4B1-B3 plasmids, probed by Western blot analysis with antibodies against FLAG and pan-PDE4B. Markers are indicated in kilodaltons. (**C**) FLAG-tagged immunoprecipitates from HEK293 cells cotransfected with DISC1 and the indicated FLAG-PDE4B1 constructs (top) probed by Western analysis with the R47 antibody to detect total DISC1 and PDE4B1-associated DISC1. For a schematic representation of PDE4B1 constructs and expression of the corresponding FLAG-tagged PDE4B1 proteins, see fig. S6. (**D**) Glutathione *S*-transferase (GST) pull-downs from lysates of HEK293 cells cotransfected with empty FLAG vector or FLAG-DISC1 and GST or GST-UCR2 plasmids, probed with antibody against FLAG to detect total DISC1 and DISC1 that specifically interacts with the UCR2 domain. (**E**) Mapping the PDE4B binding site on DISC1. ^{35}S-labeled NΔ DISC1 fragments (depicted in fig. S8) and FLAG-tagged, unlabeled PDE4B3 were synthesized by in vitro transcription-translation, mixed, and incubated for 3 hours in a binding reaction before immunoprecipitation with antibody against FLAG. Input DISC1 and PDE4B3-bound DISC1 were detected by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography. Equal capture of FLAG-tagged PDE4B3 was determined by Western analysis with antibody against FLAG (lower). Input signal represents 1/50 of total binding reaction. Markers are indicated in kilodaltons.

[1]Medical Genetics Section, Molecular Medicine Centre, University of Edinburgh, Edinburgh EH4 2XU, UK. [2]Molecular Pharmacology Group, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, Wolfson Building, University of Glasgow, Glasgow G12 8QQ, UK. [3]The Neuroscience Research Centre, Merck Sharp and Dohme, Ltd. (MSD), Terlings Park, Harlow, Essex, CM20 2QR, UK. [4]Hybrigenics S.A., 3-5 Impasse Reille–75014 Paris, France. [5]Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh EH10 5HF, UK.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: Kirsty.Millar@ed.ac.uk

Cadherin 8 (*CDH8*) gene (fig. S1) and that the 1p31.2 translocation breakpoint disrupts the B1 isoform of the phosphodiesterase 4B (*PDE4B*) gene (Fig. 1, A to C). CDH8 is a cell adhesion molecule that potentially has a neuronal function (*11*), but we focused on PDE4B for the following reasons: (i) PDE action is the sole means of inactivating intracellular adenosine 3′,5′-monophosphate (cAMP), a key second messenger involved in learning, memory, and mood (*12*–*14*). (ii) Mutations in the fruit fly *Drosophila dunce* gene, which encodes an ortholog of mammalian PDE4, cause learning and memory deficits (*13*). (iii) Mice deficient in PDE4D behave as if they are taking antidepressants (*15*). (iv) Rolipram, an antidepressant, is a selective inhibitor of PDE4 (*16*). And (v), most intriguingly, we found PDE4B (but not CDH8) to interact robustly with DISC1 in a large-scale yeast two-hybrid screen using full-length DISC1 as bait (*9*).

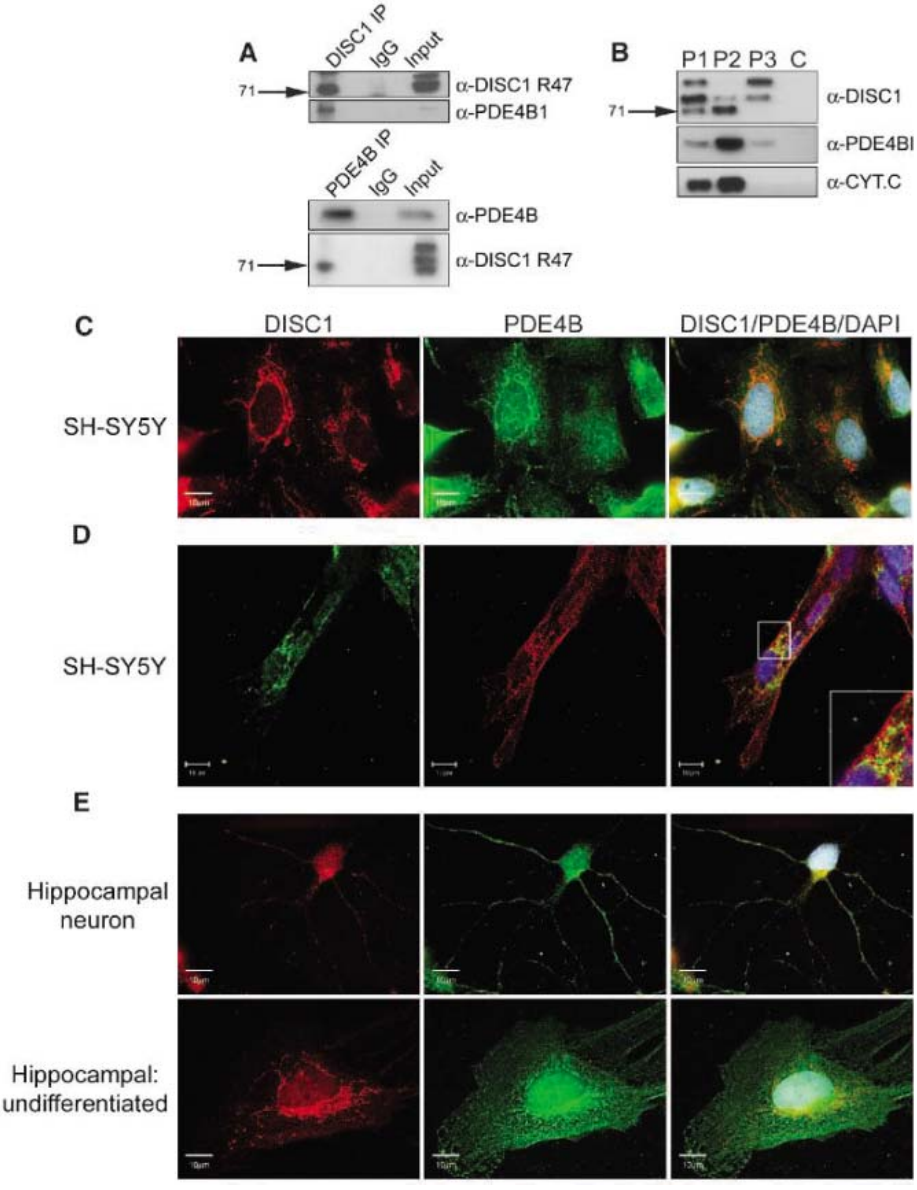


Fig. 3. Interaction of endogenous DISC1 and PDE4B in human SH-SY5Y cells. (**A**) Control immunoglobulin IgG and DISC1 immunoprecipitates (R47) from lysates of SH-SY5Y cell were probed by Western blot analysis to detect DISC1 (R47) and PDE4B1 (top), and PDE4B immunoprecipitates (pan-PDE4B) from SH-SY5Y cell extracts were probed for PDE4B (pan-PDE4B) and DISC1 (bottom). The DISC1 71-kD isoform is indicated with an arrow. (**B**) Protein extracts from SH-SY5Y subcellular fractions were probed by Western analysis with antibodies against DISC1, PDE4B1, and cytochrome c (as labeled). P1, nuclear fraction plus whole-cell debris; P2, predominantly mitochondrial; P3, other membrane elements; C, cytosolic. The DISC1 71-kD isoform is indicated with an arrow. (**C**) Immunofluorescence in SH-SY5Y human neuroblastoma cells. (Left) DISC1 (R47 antibody); middle, PDE4B (antibody against pan-PDE4B); (right) merged signal plus DAPI (nuclear DNA) stain (See also fig. S11). (**D**) Confocal immunofluorescence in differentiated SH-SY5Y human neuroblastoma cells, left to right as in (C). (Inset) Highlighted region enlarged 2.5 times. (**E**) Immunofluorescence in hippocampal primary cells, neuron and undifferentiated cell, left to right as in (C), except (left), DISC1 (C2 antibody) (See also fig. S11). Neurons were identified by using an antibody against NeuN.

To determine the effect of the t(1;16) and t(1;11) translocations on PDE4B and DISC1 expression respectively, we analyzed patient-derived lymphoblastoid cell lines. Cell lines derived from family members with or without the t(1;16) translocation revealed that PDE4B1 protein expression was reduced by ~50% in the presence of the t(1;16) translocation (Fig. 1D; fig. S2). In the case of the t(1;11) translocation, we demonstrated transcription of DISC1 from the derived chromosome 1 (fig. S3A). However, DISC1 transcript levels are reduced overall, and when we used antibody R47, which is specific for an epitope within the N terminus of DISC1 (*7*), we found no evidence [fig. S3, B to D, and (*11*)] for a putative C-terminally truncated protein (*8*, *9*). Expression of all DISC1 species was consistently reduced to about half normal levels in each of five t(1;11) cell lines from different individuals (Fig. 1E; fig. S2). These are the same DISC1 species that are detectable in human brain tissue (*7*), which indicates that proportionate reduction of DISC1 expression in brain is the most likely consequence of inheriting the translocation. The reduction in DISC1 is specific, as we see no change in the relative level of TRAX, which maps immediately 5′ of DISC1 (fig. S4, A and B). Haploinsufficiency for DISC1 in the t(1;11) translocation cases and for PDE4B in the t(1;16) translocation cases is therefore the most likely mechanistic explanation for susceptibility to schizophrenia in these individuals.

PDE4 isoforms are classified as long, short, or supershort (*17*), depending on the presence of conserved regulatory regions (UCR1 and UCR2) linked to the catalytic unit (Fig. 2A). Long isoforms uniquely express UCR1, whose phosphorylation by protein kinase A (PKA) triggers a conformational change in the UCR1 and 2 module, leading to increased catalytic activity (*17*). To determine which of the many known PDE4 isoforms could interact with DISC1, we transfected expression constructs into HEK293 cells (*11*). We analyzed the long PDE4B1 and PDE4B3 isoforms, which each contain UCR1, as well as the short PDE4B2 isoform, which does not. All three of these human isoforms co-immunoprecipitate with DISC1 (Fig. 2B), which shows that binding is not specific to one particular isoform. Indeed, DISC1 binds representative long isoforms from all four PDE4 genes (fig. S5). Removal of UCR2 abolished PDE4B1 binding to DISC1 (Fig. 2C; fig. S6). Moreover, the UCR2 domain alone was capable of binding DISC1 (Fig. 2D), which suggests that UCR2 is a specific DISC1 interaction domain.

To identify the site of PDE4 binding on DISC1, we performed binding assays after expression of DISC1 and PDE4B by in vitro transcription-translation. Full-length DISC1 (1 to 854), a C-terminal truncated DISC1 (1 to

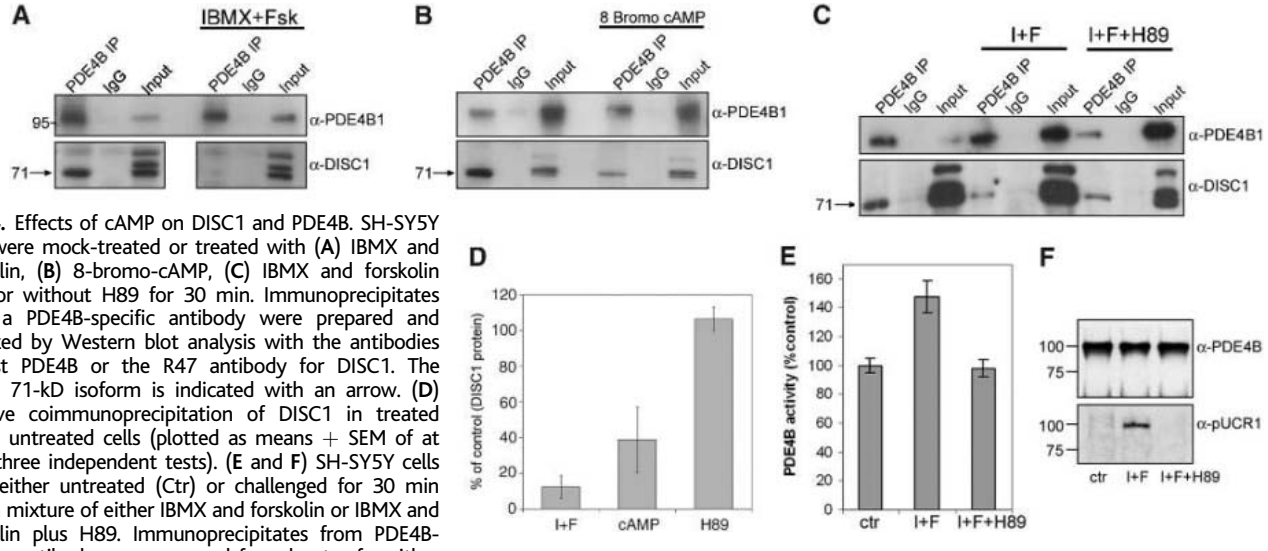


Fig. 4. Effects of cAMP on DISC1 and PDE4B. SH-SY5Y cells were mock-treated or treated with (**A**) IBMX and forskolin, (**B**) 8-bromo-cAMP, (**C**) IBMX and forskolin with or without H89 for 30 min. Immunoprecipitates from a PDE4B-specific antibody were prepared and analyzed by Western blot analysis with the antibodies against PDE4B or the R47 antibody for DISC1. The DISC1 71-kD isoform is indicated with an arrow. (**D**) Relative coimmunoprecipitation of DISC1 in treated versus untreated cells (plotted as means + SEM of at least three independent tests). (**E** and **F**) SH-SY5Y cells were either untreated (Ctr) or challenged for 30 min with a mixture of either IBMX and forskolin or IBMX and forskolin plus H89. Immunoprecipitates from PDE4B-specific antibody were prepared from lysates for either (E) PDE4 activity assays by measurement of cAMP hydrolyzing activity, or (F) Western blot analysis with a PDE4B-specific antibody or with an antiserum specific for the single PKA phosphorylation site in UCR1. Methods for obtaining these data are available online [refs. S8, S9, S13, S14, and S21 in (*11*)].

597), and the N-terminal "head" domain of DISC1 (1 to 358) were tested for their ability to bind ^{35}S-labeled PDE4B3 in vitro. All three V5-tagged DISC1 protein fragments efficiently coimmunoprecipitated PDE4B3 (fig. S7), which indicated that PDE4B binding to DISC1 is direct and that the DISC1 N-terminal domain is required for the interaction. We next generated a series of constructs that progressively delete amino acids from the N terminus of DISC1 (Fig. 2E; fig. S8) and showed that amino acids 219 to 283 are required for binding to PDE4B. DISC1 binding was restored by amino acids 180 to 250 (fig. S9).

To determine whether and where endogenous DISC1 and PDE4B interact, we performed coimmunoprecipitation and colocalization studies using conventional and confocal microscopy in the human neuroblastoma–derived cell lines SH-SY5Y and LAN5 and in primary rat hippocampal cells. DISC1 antiserum (R47) coprecipitates PDE4B1 from SH-SY5Y extracts (Fig. 3A, top), whereas pan-PDE4B, an antibody that recognizes all PDE4B isoforms, specifically coimmunoprecipitates the 71-kD DISC1 isoform (Fig. 3A, bottom). Thus, isoform PDE4B1 associates with the 71-kD isoform of DISC1. In cell fractionation studies, the PDE4B1 isoform was most abundant in the mitochondria-enriched fraction P2 (Fig. 3B), as was the 71-kD isoform of DISC1, which is also predominantly mitochondrial (*7*). PDE4B partially colocalizes with DISC1 in the mitochondria of SH-SY5Y (Fig. 3, C and D; fig. S10 and S11) and LAN5 (fig. S10) cells. In rat hippocampal primary cultures, DISC1 and PDE4B expression overlap substantially in both neurons and proliferating nonneuronal cells (Fig. 3E; fig. S10 and S11).

To further investigate DISC1-PDE4 binding, we determined the effect of elevated cAMP. Overexpression studies show that long PDE4 isoforms are activated by PKA in response to increased intracellular cAMP and, thereby, provide part of the cellular desensitization system for cAMP signaling (*18*, *19*). PKA phosphorylation of UCR1 induces conformational changes that concomitantly affect enzymatic activity and the interaction between UCR1 and UCR2 (*20*). To increase cAMP production and simultaneously to block its hydrolysis, SH-SY5Y cells were treated with forskolin plus the nonspecific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). This dramatically decreased the amount of DISC1 coprecipitating with PDE4B (Fig. 4, A and D). When cAMP levels were raised by using the cell permeable cAMP analog 8-bromo-cAMP, we similarly observed a reduction in PDE4B-DISC1 binding (Fig. 4, B and D). The action of forskolin plus IBMX was ablated when we added the PKA-specific inhibitor H89 (Fig. 4, C and D). These data indicate that the interaction between PDE4B and DISC1 is dynamic and directly influenced by altered cAMP levels through the action of PKA. Our data also indicate that DISC1 binds predominantly to the dephosphorylated, low-activity form of PDE4B, consistent with a model whereby PKA phosphorylation leads to release, from DISC1, of an activated population of PDE4B in response to elevated cAMP levels (fig. S12). To test this, we measured PDE4B cAMP hydrolyzing activity in SH-SY5Y cells and observed a 48% increase over controls, in the presence of IBMX and forskolin, that was inhibited by H89, consistent with PKA mediation of this increase (Fig. 4E). This increase in PDE4B activity is due to activation of UCR1-containing long PDE4B isoforms, because the short isoform PDE4B2 was not immunoprecipitated from SH-SY5Y cells (Fig. 4F). Using an antibody specific for the PKA-phosphorylated forms of PDE4 (*18*, *21*), we identified a 104-kD PDE4B immunoreactive species in PDE4B immunoprecipitates from cells treated with IBMX and forskolin (Fig. 4F) that is not seen in control cells, consistent with PKA phosphorylation of UCR1 as the mechanism of PDE4B activation.

Our study provides primary evidence for PDE4B as a genetic susceptibility factor for schizophrenia. We show that DISC1, an established candidate, interacts with PDE4B in a compartmentalized fashion. This interaction is dynamic and is cAMP- and PKA-dependent. We speculate that functional variation in DISC1 and/or PDE4 will modulate their interaction and affect mitochondrial cAMP catabolism with a concomitant physiological and psychiatric outcome. A unifying link between schizophrenia and bipolar affective disorder, and between DISC1 and PDE4, may occur at the cognitive level of learning and memory and at the molecular level of cAMP signaling.

References and Notes

1. P. J. Harrison, D. R. Weinberger, *Mol. Psychiatry* **10**, 40 (2005).
2. J. K. Millar *et al.*, *Hum. Mol. Genet.* **9**, 1415 (2000).
3. D. H. Blackwood *et al.*, *Am. J. Hum. Genet.* **69**, 428 (2001).
4. P. A. Thomson *et al.*, *Mol. Psychiatry* **10**, 616 (2005).
5. L. Ma *et al.*, *Genomics* **80**, 662 (2002).
6. C. P. Austin, L. Ma, B. Ky, J. A. Morris, P. J. Shugrue, *Neuroreport* **14**, 951 (2003).
7. R. James *et al.*, *Mol. Cell. Neurosci.* **26**, 112 (2004).
8. K. Miyoshi *et al.*, *Mol. Psychiatry* **8**, 685 (2003).
9. N. J. Brandon *et al.*, *Mol. Cell. Neurosci.* **25**, 42 (2004).
10. M. A. Hayashi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3828 (2005).
11. Materials and methods are available as supporting material on *Science* Online.
12. A. L. Bauman, A. S. Goehring, J. D. Scott, *Neuropharmacology* **46**, 299 (2004).
13. R. L. Davis, J. Cherry, B. Dauwalder, P. L. Han, E. Skoulakis, *Mol. Cell. Biochem.* **149**, 271 (1995).
14. R. Lamprecht, *Cell. Mol. Life Sci.* **55**, 554 (1999).
15. H. T. Zhang *et al.*, *Neuropsychopharmacology* **27**, 587 (2002).
16. J. M. O'Donnell, H. T. Zhang, *Trends Pharmacol. Sci.* **25**, 158 (2004).
17. M. D. Houslay, D. R. Adams, *Biochem. J.* **370**, 1 (2003).
18. S. J. MacKenzie *et al.*, *Br. J. Pharmacol.* **136**, 421 (2002).
19. C. Sette, M. Conti, *J. Biol. Chem.* **271**, 16526 (1996).

20. M. B. Beard *et al.*, *J. Biol. Chem.* **275**, 10349 (2000).
21. A. McCahill *et al.*, *Cell. Signal.* **17**, 1158 (2005).
22. This study was approved by the appropriate local research ethics committee. We thank M. Lai for help with the primary neuronal cultures and S. Cooper for assistance with figure production. Supported by a collaborative research agreement between Merck and Co. and the University of Edinburgh (D.J.P., D.H.R.B, W.J.M., J.K.M., B.S.P.), the Medical Research Council UK (G0100266 to D.J.P., D.H.R.B, W.J.M., G8604010 to M.D.H.) the Stanley Medical Research Institute (01-093 to J.K.M), the Scottish Hospital Endowment Research Trust (RG45/01 to B.S.P. and RG51/01 to J.K.M.), the Wellcome Trust (069300/Z/02/A to J.E.C., J.K.M. and 066717 to D.J.P., D.H.R.B., W.J.M., and others), the Chief Scientist Office of the Scottish Executive (K/MRS/50/C2789 to D.H.R.B, W.J.M., D.J.P.). W.J.M. was an MRC Clinician Scientist for part of this work.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5751/1187/DC1
Materials and Methods
Figs. S1 to S12
References and Notes

29 March 2005; accepted 3 October 2005
10.1126/science.1112915

Altered TCR Signaling from Geometrically Repatterned Immunological Synapses

Kaspar D. Mossman,[1,2,3] Gabriele Campi,[4] Jay T. Groves,[1,2,3]* Michael L. Dustin[4]*

The immunological synapse is a specialized cell-cell junction that is defined by large-scale spatial patterns of receptors and signaling molecules yet remains largely enigmatic in terms of formation and function. We used supported bilayer membranes and nanometer-scale structures fabricated onto the underlying substrate to impose geometric constraints on immunological synapse formation. Analysis of the resulting alternatively patterned synapses revealed a causal relation between the radial position of T cell receptors (TCRs) and signaling activity, with prolonged signaling from TCR microclusters that had been mechanically trapped in the peripheral regions of the synapse. These results are consistent with a model of the synapse in which spatial translocation of TCRs represents a direct mechanism of signal regulation.

Antigen recognition by T cells requires interactions between TCRs and antigenic peptides bound to major histocompatibility complex molecules (pMHCs) on antigen-presenting cells (APCs) (*1*). A coordinated recognition process ensues in which surface proteins on the T cell, along with their respective ligands, become organized into micron-scale, spatially patterned motifs known as immunological synapses (ISs) (*2*, *3*). TCRs and pMHCs occupy a region designated the central supramolecular activation cluster (c-SMAC). This is surrounded by a ring of interactions between leukocyte function-associated antigen–1 (LFA-1) on the T cell and intercellular adhesion molecule-1 (ICAM-1) on the APC, termed the peripheral supramolecular activation cluster (p-SMAC). Ultimately, an elaborate collage of adhesion, costimulatory, and signaling molecules, along with cytoskeletal attachments (*4*, *5*) and lipid rafts (*6*–*8*), organizes to sustain signaling over the hours required for full T cell activation. Although previous observations have suggested that the spatial organization of the c-SMAC is directly involved both in increasing and extinguishing TCR signaling (*9*, *10*), a direct causal relation between changes in the synaptic pattern and signaling remains to be established.

We have developed an experimental platform that enables direct manipulation of IS patterns in living T cells. A supported membrane, consisting of a continuous and fluid lipid bilayer coating a silica substrate (*11*), is used to create an artificial APC surface (*12*). Inclusion of glycosylphosphatidylinositol (GPI)–linked pMHC and ICAM-1 into the supported membrane is sufficient to enable IS formation between a T cell and the synthetic surface (*13*). This hybrid live cell–synthetic bilayer IS is illustrated schematically in Fig. 1. Fluidity is a characteristic property of supported bilayers and distinguishes them from solid and polymeric substrates. Movement within the bilayer, however, can be manipulated by fabricating geometrically defined patterns of solid-state structures on the substrate (Fig. 1) (*14*). We posited that such substrate-imposed constraints might be used to guide molecular motion in the supported bilayer and linked cell-surface receptors to generate alternatively patterned synapses.

Silica substrates displaying various configurations of chromium lines (100 nm wide and 5 nm high) were fabricated using electron-beam lithography (*15*). Supported proteolipid membranes were assembled on these substrates by vesicle fusion. As receptors on the T cell surface engage their respective ligands in the bilayer, they become subject to the geometrical restrictions on mobility imposed by the chromium lines. T cell blasts expressing the cloned AND TCR, specific for moth cytochrome c (MCC) 88-103 peptide bound to I-E^k (pMHC), were used in all IS formation experiments. In control experiments with nonactivating peptide, T cells failed to form IS patterns on substrates with chromium lines, confirming specificity of the system (fig. S1). Under conditions of specific antigen recognition, a series of IS patterns, formed under different geometries of constraint patterns, were compared with the unrestricted IS patterns (Fig. 2A). Arrays of parallel lines were found to restrict protein mobility in one dimension and skew the synaptic pattern from a circular to a rectangular shape in which a central band of TCR-pMHC is flanked by two bands of LFA-1–ICAM-1 (Fig. 2B). Multifocal ISs were observed to form in response to grid constraint

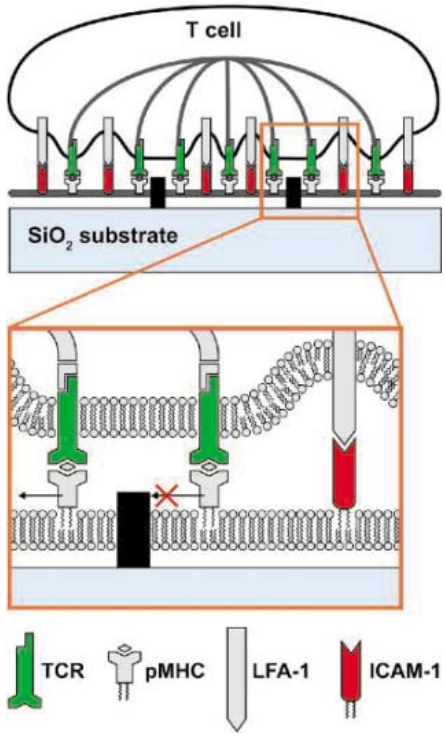


Fig. 1. Diagram of a hybrid live T cell–supported membrane junction. Receptors on the cell surface engage cognate ligands in the supported membrane and become subject to constraints on mobility imposed by physical barriers. The cytoskeleton is represented schematically to reflect the active source of central organization observed in our experiments.

[1]Biophysics Graduate Group and [2]Department of Chemistry, University of California, Berkeley, CA 94720, USA. [3]Physical Biosciences and Materials Sciences Divisions, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. [4]Department of Pathology, New York University School of Medicine, and the Program in Molecular Pathogenesis, Skirball Institute of Biomolecular Medicine, 540 First Avenue, New York, NY 10016, USA.

*To whom correspondence should be addressed. E-mail: JTGroves@lbl.gov (J.T.G.); Dustin@saturn.med.nyu.edu (M.L.D.)

patterns, which create an array of isolated membrane corrals (Fig. 2C). More elaborate constraint designs, such as a mosaic of concentric hexagonal barriers (Fig. 2D), were also used. A diverse collection of spatially mutated IS patterns were generated to investigate the effects of spatial constraints on synaptic signaling.

The chromium barriers also enabled us to provide insight into basic mechanisms of IS formation. For example, a 1-μm grid caused fragmentation of the IS into more than 100 microsynaptic TCR clusters that were stable for more than 30 min (Fig. 2E) despite the rapid TCR-pMHC off rate (~0.06 s^{-1}) (*16*). Because TCR motion can only be constrained by the grid through engagement with pMHC, the stability of corralled TCR microclusters indicates that the TCRs in each microcluster move collectively as a multimeric unit. Otherwise, individual TCRs would percolate over the barriers during disengagements from pMHC, and the stable trapping of microclusters would not be observed. The position of each TCR-pMHC microcluster within its corral revealed the direction of transport and could be used to compile a transport map of the IS (Fig. 2F). The microclusters on grids were generally "pulled" to the corner of the corral nearest the center of the IS, and images could be quantified to reveal the high degree of centralized TCR organization in frustrated synapses (fig. S2). Typically, one TCR-pMHC cluster is observed per corral for the 1-, 2-, and 5-μm square grids that were studied, suggesting that TCR clustering occurred only after pMHC engagement. Thus, if TCR were substantially preclustered, one would expect a stochastic distribution of microclusters within the corrals rather than the even distributions we observed on the 1-μm and 2-μm grids. Collectively, this set of observations supports a three-step process by which the mature IS is formed: (i) TCR engagement of pMHC, (ii) TCR-pMHC assembly into microclusters, and (iii) directed transport of microclusters to form the c-SMAC.

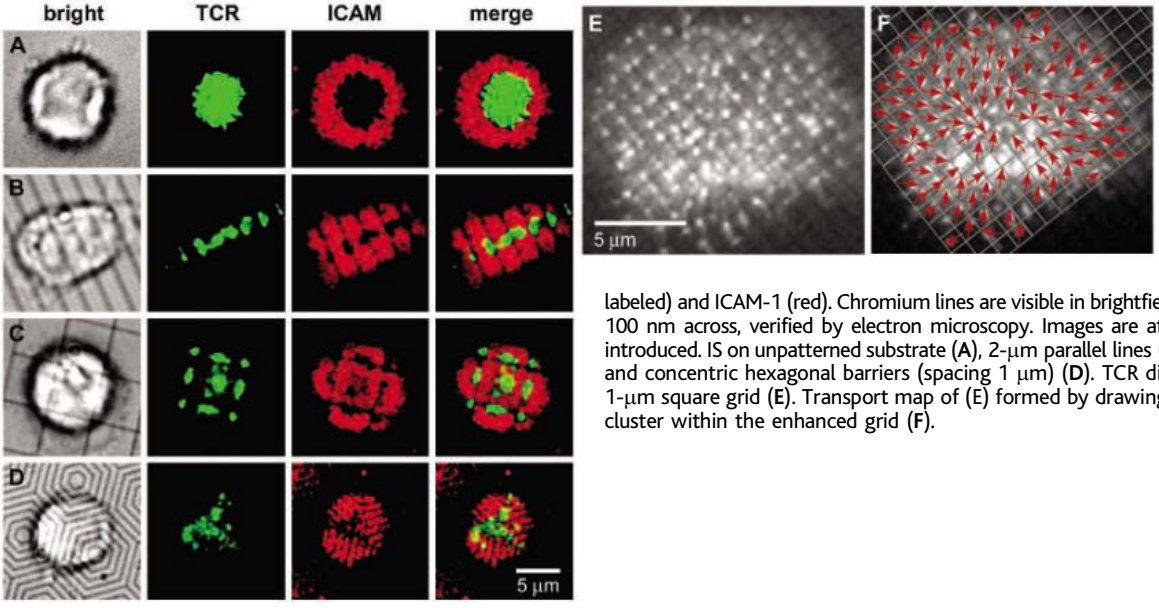


Fig. 2. Synapse formation is altered by geometrical constraints of the substrate. T cells were incubated with fluorescently labeled anti-TCR H57 Fab (green) before being introduced to supported bilayers containing GPI-linked pMHC (unlabeled) and ICAM-1 (red). Chromium lines are visible in brightfield, although they are only 100 nm across, verified by electron microscopy. Images are at 10 min after cells were introduced. IS on unpatterned substrate (**A**), 2-μm parallel lines (**B**), 5-μm square grid (**C**), and concentric hexagonal barriers (spacing 1 μm) (**D**). TCR distribution (grayscale) on 1-μm square grid (**E**). Transport map of (E) formed by drawing arrows toward the TCR cluster within the enhanced grid (**F**).

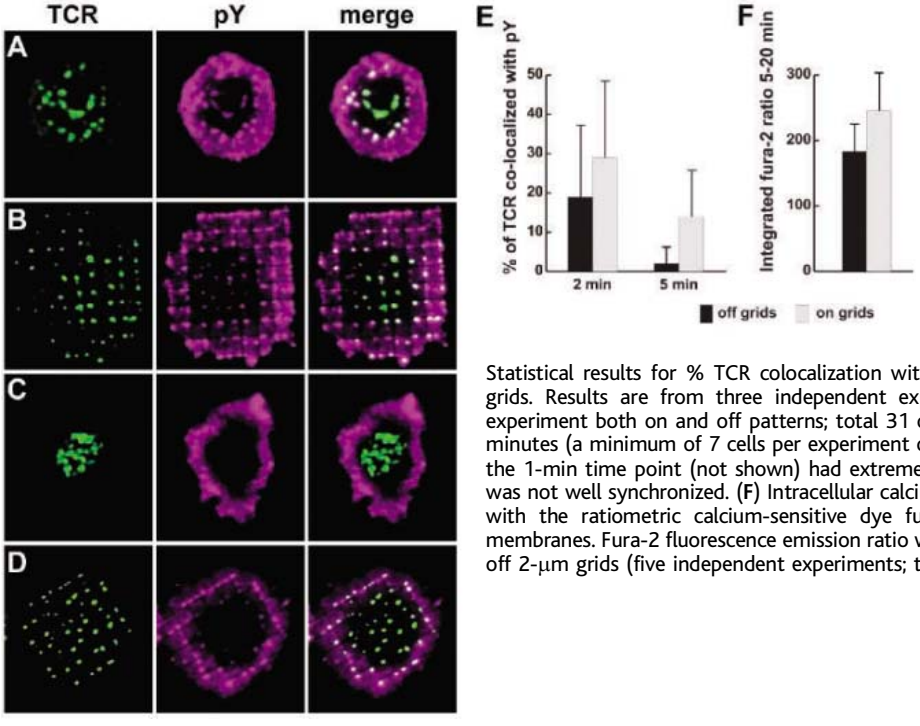


Fig. 3. TCR-specific phosphotyrosine (pY) signaling in native and repatterned synapses. T cells, which had been incubated with fluorescently labeled anti-TCR H57 Fab, were allowed to interact with pMHC-ICAM membranes for either 2 or 5 min before being fixed and stained for pY. (**A**) Synapse on unpatterned membrane at 2 min. TCR clusters are distributed, and relatively enhanced pY staining colocalizes with each cluster. The diffuse ring of pY staining in the periphery is likely associated with cortical actin. (**B**) Synapse on a 2-μm chromium grid at 2 min. (**C**) Synapse on unpatterned membrane at 5 min. (**D**) Synapse on a 2-μm chromium grid at 5 min. (**E**) Statistical results for % TCR colocalization with pY. Black, cells off pattern; gray, cells on 2-μm grids. Results are from three independent experiments at 2 min (a minimum of 9 cells per experiment both on and off patterns; total 31 on, 51 off) and four independent experiments at 5 minutes (a minimum of 7 cells per experiment on and off patterns; total 39 on, 53 off). Data from the 1-min time point (not shown) had extremely high standard deviation because cell population was not well synchronized. (**F**) Intracellular calcium is elevated in cells on grids. T cells were loaded with the ratiometric calcium-sensitive dye fura-2 and allowed to interact with pMHC-ICAM membranes. Fura-2 fluorescence emission ratio was integrated from 5 min to 20 min in cells on and off 2-μm grids (five independent experiments; total 49 on, 57 off).

Using cytoplasmic distribution of phosphorylated tyrosine (pY) residues associated with TCR clusters, we next measured signaling activity specific to each TCR cluster within constrained synapse motifs (*17*) (see also fig. S3). At early time points, pY patterns were similar in both native and repatterned synapses (Fig. 3, A and B). However, at 5 min, TCR clusters in the natively pattered IS were observed only in the c-SMAC region and had very low pY levels (Fig. 3C). In contrast, TCR clusters that had been stably restrained to the periphery of the contact area by the substrate grids retained high specific pY levels (Fig. 3D). This effect was restricted to the periphery, because TCR clusters trapped in more central regions of spatially modified synapses lost their pY signal in a time frame similar to those observed in native synapses. The duration of TCR-pY signaling thus correlated with radial position of the TCR rather than with cluster size. Overall, the extent of specific pY associated with TCR clusters above the local background was significantly greater in the IS that had been spatially constrained by the grid (Fig. 3E).

Another key measure of signaling activity is the flux of intracellular Ca^{2+} induced by TCR antigen recognition, which integrates the outputs of all TCR signaling events in the IS (*18*). The integrated Ca^{2+} response was significantly higher in cells with spatially constrained IS as compared with those with native synapses (Fig. 3F). Thus, mechanical trapping of TCR in the IS periphery augments early TCR-associated pY levels, as well as the elevation of cytoplasmic Ca^{2+}.

These experiments provide insight into how signaling is extinguished in individual TCR clusters in the IS, which may be attributed to temporal or spatial processes such as recruitment of inhibitors (*19*) or changes in the actin cytoskeleton that feed back on signaling (*20*). The hybrid live cell–supported membrane platform made it possible to physically impede receptor translocation to prevent c-SMAC formation, allowing us to resolve that radial location represents a critical parameter in the IS. In physiological terms, it is possible that some APCs may use their own cytoskeletons to restrict transport of pMHC or costimulatory molecules in a related manner. Impeding TCR cluster translocation to the c-SMAC might thus represent a means of augmenting T cell activation (*21*, *22*). Potentially, the ability to induce spatial modifications in model cell-cell interfaces could be useful in exploring spatial organization of membrane domains and proteins on the cell surface, receptor signaling activity, and cytoskeletal regulation processes.

References and Notes

1. B. P. Babbitt *et al.*, *Nature* **317**, 359 (1985).
2. D. M. Davis, M. L. Dustin, *Trends Immunol.* **25**, 323 (2004).
3. P. Friedl, J. Storim, *Trends Cell Biol.* **14**, 557 (2004).
4. V. Das *et al.*, *Immunol. Rev.* **189**, 123 (2002).
5. C. L. Fuller, V. L. Braciale, L. E. Samelson, *Immunol. Rev.* **191**, 220 (2003).
6. M. Dykstra, A. Cherukuri, H. W. Sohn, S. J. Tzeng, S. K. Pierce, *Annu. Rev. Immunol.* **21**, 457 (2003).
7. T. Harder, *Curr. Opin. Immunol.* **16**, 353 (2004).
8. R. Tavano *et al.*, *J. Immunol.* **173**, 5392 (2004).
9. K.-H. Lee *et al.*, *Science* **302**, 1218 (2003).
10. G. Campi, R. Varma, M. L. Dustin, *J. Exp. Med.* **202**, 1031 (2005).
11. E. Sackmann, *Science* **271**, 43 (1996).
12. J. T. Groves, M. L. Dustin, *J. Immunol. Methods* **278**, 19 (2003).
13. A. Grakoui *et al.*, *Science* **285**, 221 (1999).
14. J. T. Groves, N. Ulman, S. G. Boxer, *Science* **275**, 651 (1997).
15. B. L. Jackson, J. T. Groves, *J. Am. Chem. Soc.* **126**, 13878 (2004).
16. M. M. Davis *et al.*, *Annu. Rev. Biochem.* **72**, 717 (2003).
17. K. H. Lee *et al.*, *Science* **295**, 1539 (2002).
18. D. J. Irvine, M. A. Purbhoo, M. Krogsgaard, M. M. Davis, *Nature* **419**, 845 (2002).
19. P. Fischer *et al.*, *Biochem. J.* **378**, 449 (2004).
20. J. Xu *et al.*, *Cell* **114**, 201 (2003).
21. S. Y. Tseng, M. Lu, M. L. Dustin, *J. Immunol.*, in press.
22. M. M. Al-Alwan, G. Rowden, T. D. Lee, K. A. West, *J. Immunol.* **166**, 1452 (2001).

23. We thank A. L. DeMond and R. Varma for helpful discussions. Supported by Boehringer Ingelheim (G.C.) and NIH grant GM64900.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1191/DC1
Materials and Methods
SOM Text
Figs. S1 to S4

23 August 2005; accepted 24 October 2005
10.1126/science.1119238

Regulation of Yeast Replicative Life Span by TOR and Sch9 in Response to Nutrients

Matt Kaeberlein,[1]* R. Wilson Powers III,[1] Kristan K. Steffen,[2] Eric A. Westman,[2] Di Hu,[2] Nick Dang,[2] Emily O. Kerr,[2] Kathryn T. Kirkland,[2] Stanley Fields,[1,3] Brian K. Kennedy[2]*

Calorie restriction increases life span in many organisms, including the budding yeast *Saccharomyces cerevisiae*. From a large-scale analysis of 564 single-gene–deletion strains of yeast, we identified 10 gene deletions that increase replicative life span. Six of these correspond to genes encoding components of the nutrient-responsive TOR and Sch9 pathways. Calorie restriction of *tor1Δ* or *sch9Δ* cells failed to further increase life span and, like calorie restriction, deletion of either *SCH9* or *TOR1* increased life span independent of the Sir2 histone deacetylase. We propose that the TOR and Sch9 kinases define a primary conduit through which excess nutrient intake limits longevity in yeast.

Calorie restriction (CR) is the only intervention known to increase life span in yeast, worms, flies, and mammals, but the molecular mechanism for this phenomenon has not been clear. In yeast, CR due to reduced glucose concentration of the culture medium increases replicative life span (the number of daughter cells produced by a given mother cell before senescence) by 20 to 40% (*1*–*3*). This increased life span has been attributed to activation of Sir2 (*1*), a histone deacetylase that is dependent on NAD (the oxidized form of nicotinamide adenine dinucleotide) (*4*) and that promotes longevity by inhibiting the formation of extrachromosomal ribosomal DNA (rDNA) circles (ERCs) in the nucleolus (*5*). Recently, however, the link between Sir2 and CR has been called into question with the discovery that Sir2 is not required for life-span extension by CR (*3*).

To identify genes that regulate longevity in the budding yeast, a large-scale analysis of replicative life span was conducted with the *MATα* haploid open reading frame (ORF) deletion collection, a set of ~4800 single-gene–deletion strains (*6*). Because replicative life-span analysis requires labor-intensive micromanipulation of daughter cells from mother cells, fewer than 80 different genes have been previously examined for their effect on replicative life span (*7*). Here we examined the replicative aging properties of 564 single-gene–deletion strains (Fig. 1A; table S1).

An iterative method was designed to identify ~95% of strains with mean replicative life span at least 30% longer than wild type (*8*). For each single-gene–deletion strain, replicative life span was initially determined for five individual mother cells. If the mean life span was less than 26 generations, the strain was classified as not-long-lived (NLL). This lower cutoff value is predicted to result in misclassification of a long-lived strain less than 5% of the time (fig. S1). If the mean life span was less than 20, the strain was classified as short-lived (SL). If the mean life span was greater than 36, the strain was putatively classified as long-lived (LL),

[1]Departments of Genome Sciences and Medicine, [2]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA. [3]Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195 USA.

*To whom correspondence should be addressed. E-mail: kaeber@u.washington.edu (M.K.); bkenn@u.washington.edu (B.K.K.)

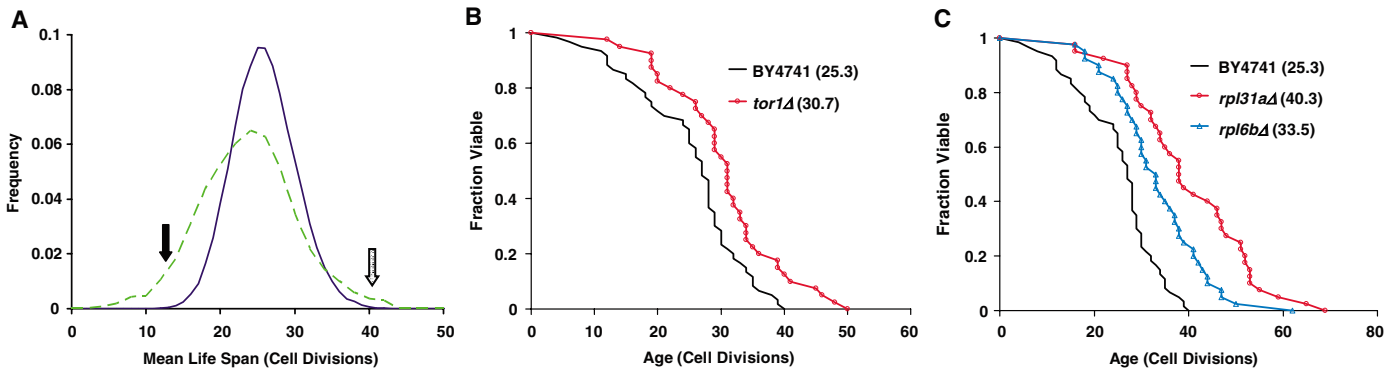


Fig. 1. TOR activity is an important modifier of yeast longevity. (**A**) The distribution of observed strain mean life spans for 564 single-gene–deletion mutants (broken line) shows an overrepresentation of short-lived (dark arrow) and long-lived (light arrow) mutants relative to expected mean life-span distribution (solid lines) for wild-type cells of the same sample size (n = 5). (**B**) Deletion of *TOR1* increases life span. (**C**) Deletion of either *RPL31A* or *RPL6B*, ribosomal proteins transcriptionally regulated by TOR, increases life span. Mean life spans are shown in parentheses.

and an additional 10 cells were examined. This upper cutoff value is predicted to result in misclassification of a strain with wild-type life span less than 2% of the time. For the remaining strains with a five-cell mean life span between 26 and 36 generations, an additional five cells were analyzed (one iteration), and the same classification scheme was applied. This process was repeated until every strain was either classified as SL, NLL, or LL or until replicative life span had been determined for a minimum of 15 cells for each unclassified strain. The replicative life-span data for strains from which at least 15 mother cells had been assayed were compared with cell life-span data from wild-type mothers, matched by experiment, by using a Wilcoxon rank-sum test to generate a P value. Strains with $P \leq 0.1$ were classified as LL, and strains with $P > 0.1$ were classified as having a life span not significantly extended (NSE). Of the 564 strains analyzed, 114 were classified as SL, 254 as NLL, 152 as NSE, and 44 as LL. Although nearly 20% of the gene deletions resulted in a significantly shortened life span, relatively few of these are likely to represent a true premature aging phenotype, because dysregulation of many different cellular processes will decrease fitness and longevity (*9*). For this reason, we focused on genes that, when deleted, resulted in increased replicative life span, reasoning that the proteins encoded by these genes must impede the normal aging process.

Of the 44 single-gene–deletion strains initially classified as LL, 13 result in a significant increase in replicative life span (Table 1). Verification was accomplished by determining the replicative life span for the corresponding gene deletion strain from the haploid *MAT***a** deletion collection and, in select cases, by generating a new deletion allele in the parental BY4742 strain. Of the 13 genes, *FOB1* served as a proof of principle that our method can identify a true-positive aging gene, because deletion of *FOB1* is known to increase life span by reducing the formation of ERCs (*10*). In two cases, gene deletions conferring increased life span occurred in overlapping ORFs encoded on opposite strands (*REI1* contains *YBR266C*; *IDH2* overlaps *YOR135C*), and longevity was comparable for overlapping deletion pairs (table S2). The identification of two different overlapping gene pairs from this screen suggests that a high fraction of true-positive genes were successfully identified.

The most striking feature of the 10 (excluding the overlapping dubious ORFs and *FOB1*) newly identified aging genes is that 6 are implicated in the TOR signaling pathway. TOR proteins are highly conserved from yeast to humans and regulate multiple cellular processes in response to nutrients, including cell size, autophagy, ribosome biogenesis and translation, carbohydrate and amino acid metabolism, stress response, and actin organization (*11*). Yeast has two TOR proteins, Tor1 and Tor2. Tor2 is essential and, therefore, not represented in the deletion collection. Deletion of *TOR1* was identified from this screen and found to increase both mean and maximum life span by ~20% (Fig. 1B). Two downstream targets of Tor1 and Tor2 were also identified: Ure2, which regulates activity of the nitrogen-responsive transcription factor Gln3, and Rom2, a proposed activator of protein kinase C (*12*, *13*). Deletion of three genes that are transcriptionally up-regulated by TOR increased life span: *YBR238C*, a gene of unknown function (*14*), and *RPL31A* and *RPL6B*, encoding two components of the large ribosomal subunit (Fig. 1C). Not all TOR-regulated ribosomal protein gene deletions examined conferred increased life span. Unlike the case in most multicellular eukaryotes, many of the ribosomal protein genes are duplicated in yeast (e.g., *RPL31A* and *RPL31B*), which allows for viable deletion of either paralog (but not both simultaneously). The relative importance of each paralog for ribosomal function, perhaps reflecting differential expression levels, may determine the longevity phenotype on deletion, with the gene coding for the more abundant member of the pair more likely to influence life span. Consistent with this idea, *rpl31aΔ* mother cells are long-lived and slow growing, whereas *rpl31bΔ* mother cells are not (fig. S2).

Table 1. Long-lived deletion strains. From a screen of 564 single-gene–deletion strains, 13 genes were found to increase replicative life span when deleted. GDP, guanosine diphosphate; GTP, guanosine 5′-triphosphate; PI3-like kinase, a kinase like phosphatidylinositol 3-kinase.

Deletion strain	Protein function
bre5Δ	Ubiquitin protease
fob1Δ	rDNA replication fork barrier protein
idh2Δ	Isocitrate dehydrogenase
rei1Δ	Protein of unknown function with similarity to human ZPR9
rom2Δ	GDP-GTP exchange factor for Rho1p
rpl31aΔ	Ribosomal protein L31
rpl6bΔ	Ribosomal protein L6
tor1Δ	PI3-like kinase involved in regulation of cell growth
ure2Δ	Regulator of nitrogen catabolite repression
ybr238cΔ	Protein of unknown function
ybr255wΔ	Protein of unknown function
ybr266cΔ	Hypothetical ORF overlapping *REI1*
yor135cΔ	Hypothetical ORF overlapping *IDH2*

Protein kinase A (PKA) and Sch9 are nutrient-responsive protein kinases that modulate replicative aging in yeast (*1*, *15*). Mutations that decrease PKA activity increase replicative life span and have been suggested as genetic models of CR (*1*, *3*). TOR is thought to act both upstream and parallel to PKA, whereas Sch9 is thought to act in a pathway parallel to PKA and TOR (*16*, *17*). TOR, PKA, and Sch9 regulate the expression of common downstream targets, including ribosomal proteins, such as Rpl31a and Rpl6b (*18*, *19*). CR of *tor1Δ* or *sch9Δ* cells failed to significantly increase the life span of these long-lived mutants (Fig. 2, A and B), which indicates that, similar to PKA, Sch9 and TOR are targets of CR in yeast. CR by growth

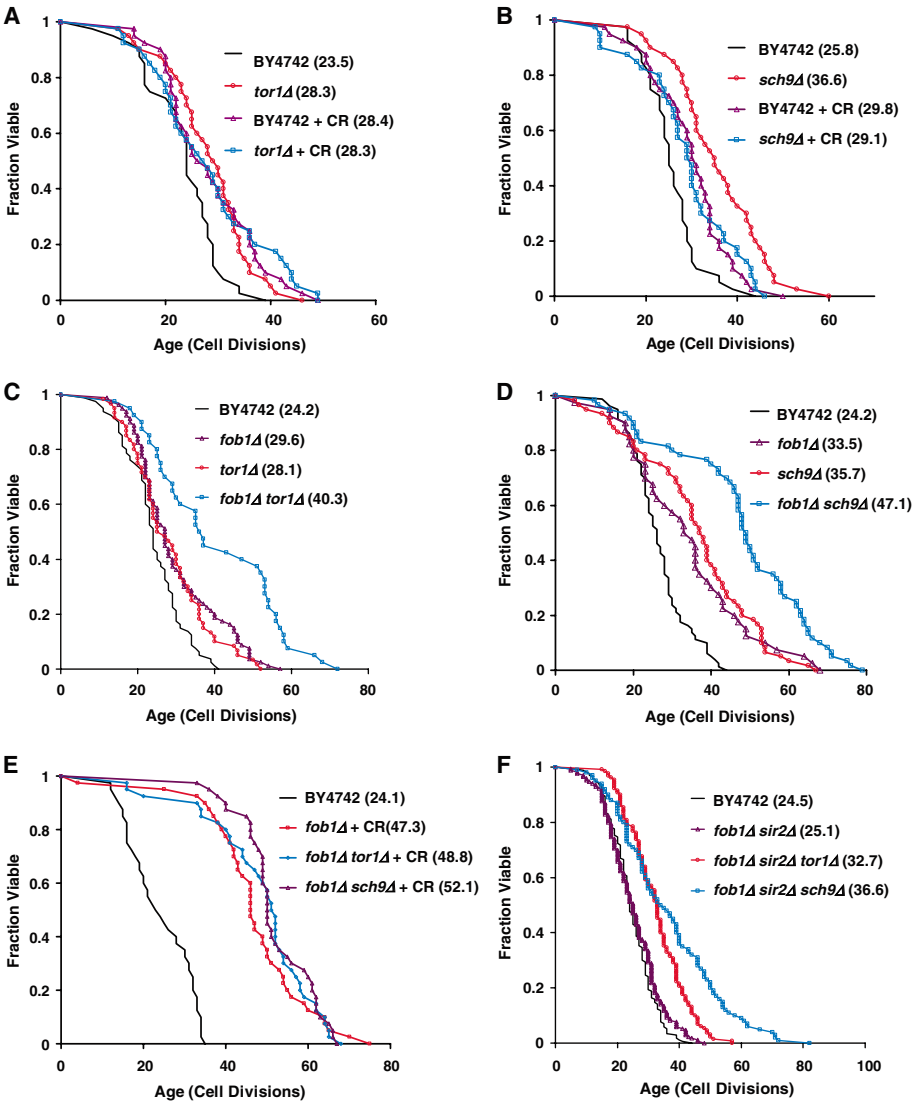


Fig. 2. *TOR1* or *SCH9* deletion mutants are genetic mimics of CR. (**A**) CR fails to further increase the life span of cells lacking *TOR1*. (**B**) CR fails to further increase the life span of cells lacking *SCH9*. (**C**) Deletion of *TOR1* increases life span additively with deletion of *FOB1*. (**D**) Deletion of *SCH9* increases life span additively with deletion of *FOB1*. (**E**) Deletion of either *TOR1* or *SCH9* fails to increase the life span of calorie-restricted *fob1Δ* cells. (**F**) Deletion of either *TOR1* or *SCH9* increases the life span of *sir2Δ fob1Δ* double-mutant cells. Mean life spans are shown in parentheses.

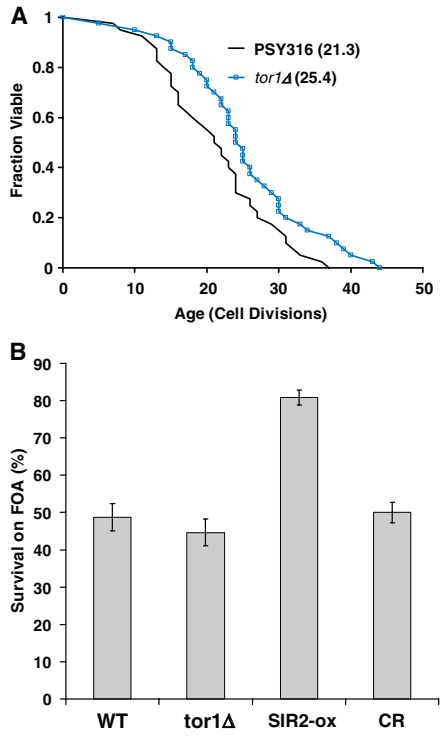


Fig. 3. Decreased TOR activity, like CR, is a strain-independent modifier of replicative life span. (**A**) Deletion of *TOR1* increases life span in the PSY316 background. Mean life spans are shown in parentheses. (**B**) Deletion of *TOR1* and CR have no effect on Sir2-dependent silencing of a telomeric *URA3* marker gene, as measured by survival in the presence of 5-FOA, in the PSY316 background. An extra copy of Sir2 (SIR2-ox) increases silencing of telomeric *URA3*. WT is wild-type.

on low glucose, or mutations resulting in decreased PKA activity, increase life span additively with deletion of *FOB1* (*3*). Deletion of *TOR1* or deletion of *SCH9* also resulted in an additive increase in life span when combined with deletion of *FOB1* (Fig. 2, C and D). The already long life span of the *sch9Δ fob1Δ* or *tor1Δ fob1Δ* mother cells was not further increased by CR (Fig. 2E). Life-span extension by CR also occurs independently of Sir2, as long as ERC formation is kept low through deletion of *FOB1* (*3*). Deletion of either *TOR1* or *SCH9* also increased the life span of *sir2Δ fob1Δ* cells (Fig. 2F). These epistasis experiments suggest that decreased activity of the nutrient-responsive kinases Sch9 and TOR in response to CR results in increased replicative life span in yeast.

Life-span extension by CR in yeast was initially characterized in the short-lived strain background PSY316 (*1*). PSY316 is unique among yeast strains used for longevity studies in that, although CR increases life span by 30 to 40%, deletion of *FOB1* or overexpression of *SIR2* fails to result in increased life span (*20*). To determine whether TOR activity is a general or strain-specific determinant of replicative life span, we examined the effect of *TOR1* deletion on life span and Sir2 activity in the PSY316 background. Deletion of *TOR1* significantly increased life span in PSY316, but had no effect on Sir2-dependent silencing at telomeres, similar to the effect of CR by growth on low glucose (Fig. 3, A and B). Thus, like CR, decreased TOR activity is a strain-independent mechanism to achieve enhanced longevity in yeast.

TOR activity is a primary determinant of replicative aging in yeast, and genetic analysis indicates that Sir2-independent life-span extension by CR is mediated by reduced signaling through TOR, Sch9, and PKA, resulting in down-regulation of ribosome biogenesis. Recently, an alternative model has suggested that Sir2-independent CR is caused by decreased ERC formation, resulting from nuclear relocalization and activation of the Sir2 homolog Hst2 (*21*). However, as long as ERC formation is maintained at a low level, CR increases life span to a greater extent in cells lacking Sir2 than in cells where Sir2 is present, seemingly inconsistent with Hst's simply playing a role redundant to Sir2's. CR increases life span additively with deletion of *FOB1*, which suggests a mechanism for CR that is independent of ERCs. ERCs also affect aging only in yeast, whereas the longevity-promoting role of CR has been evolutionarily conserved.

Decreased activity of TOR and Sch9 orthologs increases life span in *Caenorhabditis elegans* (*22*, *23*) and *Drosophila melanogaster* (*24*), as does mutation of the TOR-regulated S6 kinase (*24*), which promotes ribosomal protein maturation in multicellular eukaryotes. Therefore, the data presented here are consistent

with a model whereby CR increases life span through a highly conserved, Sir2-independent signaling network from nutrients to ribosomes.

References and Notes

1. S. J. Lin, P. A. Defossez, L. Guarente, *Science* **289**, 2126 (2000).
2. S. J. Lin *et al.*, *Nature* **418**, 344 (2002).
3. M. Kaeberlein, K. T. Kirkland, S. Fields, B. K. Kennedy, *PLoS Biol.* **2**, E296 (2004).
4. S. Imai, C. M. Armstrong, M. Kaeberlein, L. Guarente, *Nature* **403**, 795 (2000).
5. M. Kaeberlein, M. McVey, L. Guarente, *Genes Dev.* **13**, 2570 (1999).
6. E. A. Winzeler *et al.*, *Science* **285**, 901 (1999).
7. M. Kaeberlein, K. T. Kirkland, S. Fields, B. K. Kennedy, *Mech. Ageing Dev.* **126**, 491 (2005).
8. Materials and methods are available as supporting material on *Science* Online.
9. M. Kaeberlein, B. K. Kennedy, *Mech. Ageing Dev.* **126**, 17 (2005).
10. P. A. Defossez *et al.*, *Mol. Cell* **3**, 447 (1999).
11. T. Schmelzle, M. N. Hall, *Cell* **103**, 253 (2000).
12. T. Beck, M. N. Hall, *Nature* **402**, 689 (1999).
13. S. B. Helliwell, I. Howald, N. Barbet, M. N. Hall, *Genetics* **148**, 99 (1998).
14. A. F. Shamji, F. G. Kuruvilla, S. L. Schreiber, *Curr. Biol.* **10**, 1574 (2000).
15. P. Fabrizio, S. D. Pletcher, N. Minois, J. W. Vaupel, V. D. Longo, *FEBS Lett.* **557**, 136 (2004).
16. S. A. Zurita-Martinez, M. E. Cardenas, *Eukaryot. Cell* **4**, 63 (2005).
17. I. Pedruzzi *et al.*, *Mol. Cell* **12**, 1607 (2003).
18. P. Jorgensen *et al.*, *Genes Dev.* **18**, 2491 (2004).
19. D. E. Martin, A. Soulard, M. N. Hall, *Cell* **119**, 969 (2004).
20. M. Kaeberlein *et al.*, *J. Biol. Chem.* **280**, 17038 (2005).
21. D. W. Lamming *et al.*, *Science* **309**, 1861 (2005); published online 28 July 2005 (10.1126/science.1113611).
22. M. Hertweck, C. Gobel, R. Baumeister, *Dev. Cell* **6**, 577 (2004).
23. T. Vellai *et al.*, *Nature* **426**, 620 (2003).
24. P. Kapahi *et al.*, *Curr. Biol.* **14**, 885 (2004).

25. We thank G. Martin and P. Rabinovitch for advice and helpful discussion. M.K. is supported by NIH training grant P30 AG013280 and by the Ellison Medical Foundation. This work was funded by awards to B.K. from the University of Washington Nathan Shock Center of Excellence for the Basic Biology of Aging, the American Federation for Aging Research, and the Ellison Medical Foundation. S.F. is an investigator of the Howard Hughes Medical Institute. B.K. is a Searle scholar. M.K. cofounded and served as vice president, Biology of Aging Research for Longenity, Inc., until May 2003.

Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5751/1193/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References and Notes

31 May 2005; accepted 5 October 2005
10.1126/science.1115535

Golgi Duplication in *Trypanosoma brucei* Requires Centrin2

Cynthia Y. He, Marc Pypaert, Graham Warren*

Centrins are highly conserved components of the centrosome, which in the parasitic protozoan *T. brucei* comprises the basal body and nucleates the flagellum used for locomotion. Here, we found TbCentrin2 in an additional bi-lobed structure near to the Golgi apparatus. One lobe was associated with the old Golgi, and the other became associated with the newly forming Golgi as the cell grew. Depletion of TbCentrin1 inhibited duplication of the basal body, whereas depletion of TbCentrin2 also inhibited duplication of the Golgi. Thus, a Centrin2-containing structure distinct from the basal body appears to mark the site for new Golgi assembly.

Organelle duplication helps to ensure propagation through successive generations. For the Golgi apparatus, a number of models have been put forward, which differ as to the role played by the old Golgi in the construction of the new (*1*). What has not been addressed is the mechanism that determines the location for assembly of the new Golgi. In the budding yeast, *Pichia pastoris*, this location appears to be random, based on the probability of components reaching a critical mass for assembly (*2*). In several parasitic protozoa and in other protists, however, this location seems to be defined (*1*, *3*). In *T. brucei*, for example, there is a single Golgi, and a new copy is assembled at a fixed distance away (*4*). This new assembly site is somehow related to the new basal body (*5*); inhibition of basal-body segregation inhibits that of the Golgi (*4*) and inhibits division of the replicated kinetoplast, which contains all of the mitochondrial DNA (*6*). Basal bodies in particular and centrosomes in general have been implicated in the biogenesis of a number of membrane-bound organelles, in a variety of organisms (*6*, *7*), prompting us to study further their role in Golgi duplication.

Centrins are Ca^{2+}-binding proteins that are highly conserved and essential components of all centrosomes (*8*). The monoclonal antibody, 20H5, raised against *Chlamydomonas reinhardtii* Centrin (*9*), labels centrosomes in a wide range of organisms. It can also stain the basal bodies in *T. brucei* at different stages of the cell cycle (Fig. 1). The basal bodies are closely associated with the kinetoplast and mediate the division of the replicated kinetoplast (*6*).

The 20H5 antibody stained an additional, bi-lobed structure (Fig. 1). Early in the cell cycle, the old Golgi was adjacent to one lobe (Fig. 1A), whereas the new Golgi was later seen to be adjacent to the other, more posterior lobe (Fig. 1B), suggesting that it might be marking the site for new assembly. As the new Golgi grew and increasingly separated from the old (Fig. 1C), the bi-lobed structure itself duplicated, so that one remained with the old Golgi and one with the new (Fig. 1, D and E). This occurred at about the same time as the division of the replicated kinetoplast (Fig. 1E).

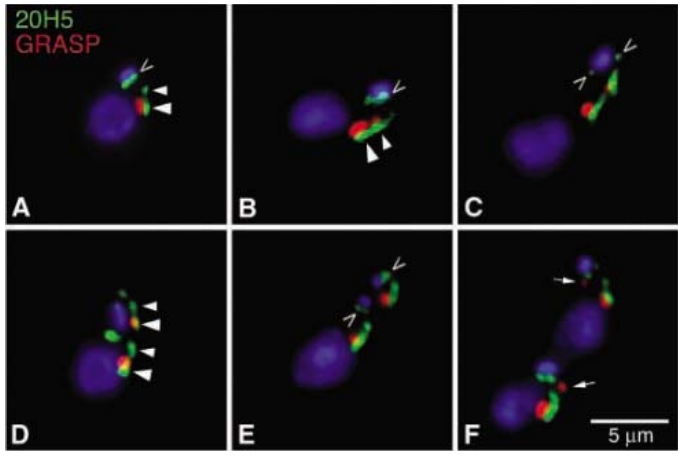


Fig. 1. A Centrin-containing structure associated with the Golgi. Gallery of images through the cell cycle of cells triple labeled for Golgi [anti–Golgi Reassembly Stacking Protein (GRASP), red], Centrin (20H5, green), and DNA [4′,6′-diamidino-2-phenylindole (DAPI), blue]. Basal bodies [open arrowheads (**A** and **B**)] underwent duplication (**C** and **D**) and mediated the division of the replicated kinetoplast (**E**). Centrin associated with the Golgi was present in a bi-lobed structure (solid arrowheads), with one lobe near to the old Golgi (A) and the other more toward the posterior of the parasite, marking the site where the new Golgi was undergoing assembly [compare (A) and (B)]. Increasing separation of the old and new Golgi (C) was accompanied by duplication of this bi-lobed structure [(C) to (E)] at about the same time as the replicated kinetoplast divided (E). (**F**) Just before cytokinesis, additional Golgi (arrows) appeared that were not associated with Centrin. Scale bar, 5 μm.

Department of Cell Biology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: graham.warren@yale.edu

At later times in the cell cycle, when additional Golgi appeared (Fig. 1F, arrows) (*4*), they were not associated with this new structure. Though the function of these additional Golgi is unclear, the data do suggest that they are not part of the core duplication process.

There are five putative Centrins in *T. brucei* (*10*). TbCentrins1 to 4 are the most homologous, by both sequence and phylogenetic analysis, to the *C. reinhardtii* Centrin used to raise the 20H5 monoclonal antibody. Each of these was tagged with the yellow fluorescent protein (YFP) and stably expressed, followed by immunoblot analysis. Only TbCentrin1 and TbCentrin2 were recognized by the 20H5 antibody (fig. S1). Whereas TbCentrin1 was localized exclusively to the basal body (Fig. 2A), TbCentrin2 was also associated with the Golgi (Fig. 2, B and C). This association did not depend on the position of the YFP, because tagging the N terminus gave the same pattern at all stages of the cell cycle and was identical to that seen with 20H5 (compare Fig. 1, A to F, with Fig. 2, F to J). Endogenous TbCentrin2 could not be examined by immunofluorescence microscopy because the polyclonal antibodies proved to be too insensitive. Both alleles were thus replaced by TbCentrin2 tagged at the C terminus with the short, viral, BB2 tag (*11*). The cells had the same growth characteristics as the parental line, indicating that this construct was fully functional. The location of TbCentrin2-BB2 was also identical to that identified by 20H5 and both of the YFP constructs (compare Fig. 2, D and E, with Fig. 1, A to F, and Fig. 2, F to J). Thus, a pool of TbCentrin2 defines a bi-lobed, Golgi-associated structure apparently distinct from the basal bodies.

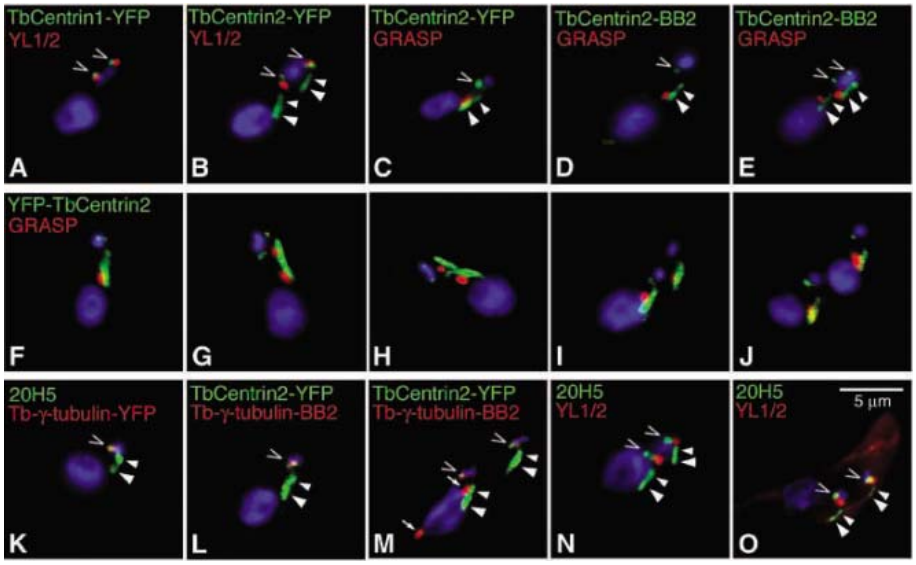


Fig. 2. TbCentrin2 associated with the Golgi. (**A** to **J**) Cells stably expressing the indicated TbCentrin constructs (green) were labeled for tyrosinated α-tubulin (YL1/2) (red) in (A) and (B) or GRASP (red) in (C) to (J), and DNA (DAPI, blue). TbCentrin2, tagged with BB2 [(D) and (E)], replaced both of the endogenous alleles. TbCentrin1 localized to the basal body alone [open arrowheads in (A)], whereas TbCentrin2 also localized to the Golgi [solid arrowheads in (B) to (E)] throughout the cell cycle [(F) to (J)]. (**K** to **O**) Cells were labeled for Centrin (green) with the use of 20H5 [(K), (N), and (O)] or by stable expression of TbCentrin2-YFP [(L) and (M)]. Centrin associated with the Golgi (solid arrowheads) did not colocalize with the basal-body markers (red), gamma-tubulin [tagged with YFP in (K) or BB2 in (L) and (M)] or tyrosinated α-tubulin [YL1/2 in (N) and (O)]. Open arrowheads mark the basal bodies, whereas arrows mark the mitotic spindle poles. Scale bar, 5 μm.

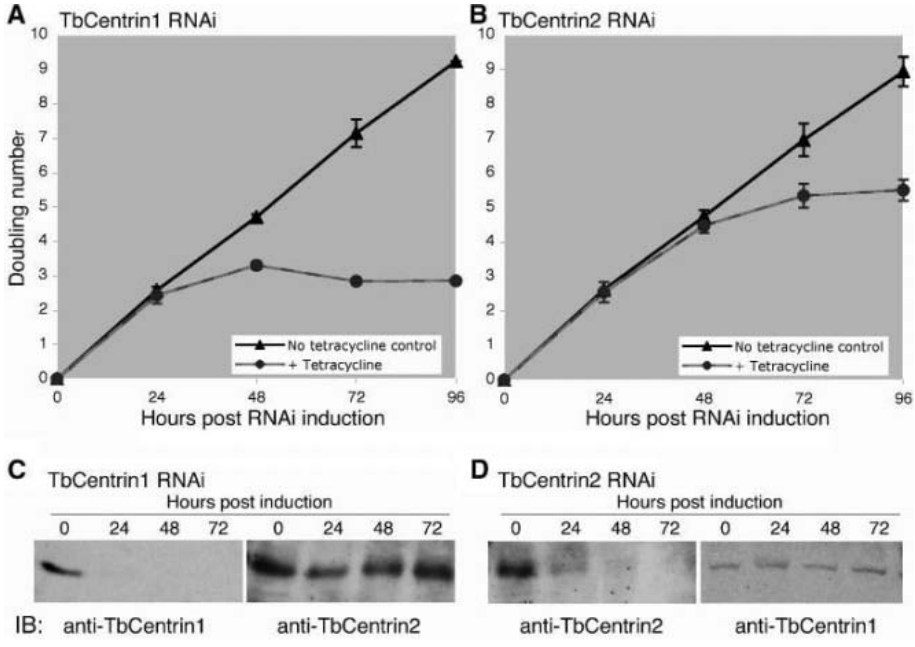


Fig. 3. RNAi knockdown of TbCentrin1- or TbCentrin2-inhibited cell growth. Cells were grown in the absence or presence of tetracycline to induce RNAi, and samples were taken for (**A** and **B**) counting (results presented as mean ± SD, $n = 3$) and (**C** and **D**) immmunoblotting (IB) to assess levels of TbCentrin1 and TbCentrin2 in fractionated extracts. Cessation of growth occurred earlier in cells depleted of TbCentrin1, consistent with the more rapid loss of protein. Loss was specific; i.e., depletion of TbCentrin1 had no effect on levels of TbCentrin2, and vice versa.

To distinguish this structure from basal bodies in particular and centrosomes in general, we tested other markers. Gamma-tubulin is found in all centrosomes (*12*) and is associated with the Golgi in mammalian cells (*13*). None could be detected, however, in the bi-lobed structure when YFP-tagged gamma-tubulin was stably expressed. It was only found in the basal bodies (Fig. 2K). Gamma-tubulin is known to be present in other structures (*14*) and the YFP tag might thus have restricted its distribution. Gamma-tubulin was thus tagged with BB2 and stably expressed. Additional staining of the mitotic spindle poles during mitosis was then observed (Fig. 2, L and M), as expected (*14*), but there was no staining of the bi-lobed structure. There was also no staining using the YL1/2 antibody (Fig. 2, N and O), which recognizes tyrosinated α-tubulin. This is found in the basal bodies throughout the cell cycle and, during certain stages of the cell cycle, in the flagellar axoneme and the posterior third of the subpellicular microtubules that contain newly synthesized microtubules (*15*).

To test whether TbCentrin2 plays a functional role in Golgi duplication, the inducible and inheritable RNA interference (RNAi) system in *T. brucei* (*16*) was exploited so as to deplete either TbCentrin1 or TbCentrin2. In the presence of tetracycline, to induce expression of the double-stranded RNA, the cells stopped growing in the absence of either Centrin (Fig. 3, A and B), consistent with the effects of Centrin depletion in other organisms (*17*–*19*). Polyclonal antibodies directed against

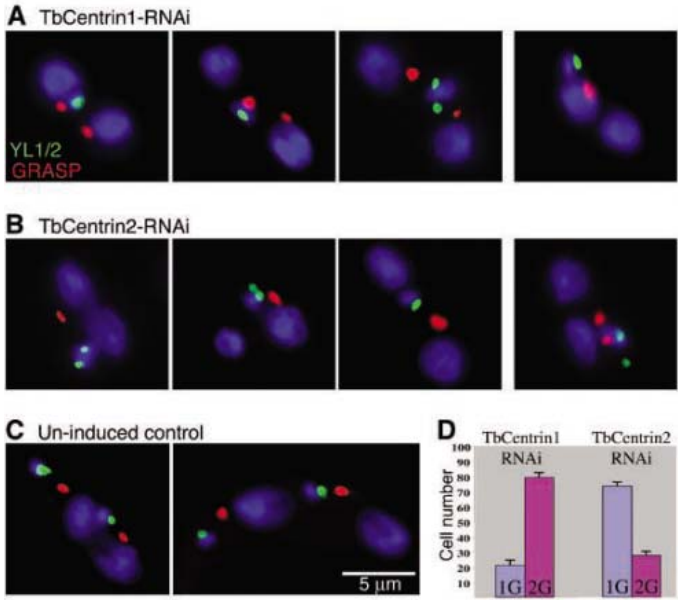


Fig. 4. RNAi knockdown of TbCentrin2 but not TbCentrin1 inhibited Golgi duplication. Cells were fixed at (**A**) 24 hours (TbCentrin1) or (**B**) 48 hours (TbCentrin2) after induction of RNAi, and triple labeled for GRASP (red), tyrosinated α-tubulin (YL1/2) (green) and DNA (DAPI, blue). (**C**) Uninduced cells were used as controls. (**D**) Cells late in the cell cycle, containing two nuclei and one kinetoplast, were imaged and the number of Golgi counted (results presented as mean percentage + SD, $n = 3$). The depletion of TbCentrin1 inhibited complete duplication of the basal body but not the Golgi, whereas depletion of TbCentrin2 inhibited duplication of both. Scale bar, 5 μm.

either TbCentrin1 or TbCentrin2 (fig. S1) were used to monitor the levels. Depletion of TbCentrin1 had no effect on the levels of TbCentrin2 and vice versa (Fig. 3, C and D).

In both cases, depletion led to an inhibition of basal-body duplication, and hence kinetoplast division, but had no effect on nuclear division. At early times, this resulted in cells containing one kinetoplast and two nuclei. At later times, these became multinucleate because cytokinesis was also inhibited. To facilitate analysis, cells containing one kinetoplast and two nuclei were counted. Cells depleted of TbCentrin1 stopped growing earlier than those depleted of TbCentrin2, the likely consequence of TbCentrin1 being depleted more rapidly than TbCentrin2 (compare Fig. 3, C and D). We thus chose to look at TbCentrin1-depleted cells at 24 hours and TbCentrin2-depleted cells at 48 hours postinduction. At these time points, the cells containing one kinetoplast and two nuclei constituted ~10% of the total population.

Depletion of either TbCentrin1 or TbCentrin2 had a marked effect on the duplication of the basal bodies (Fig. 4, A and B). Most cells had one labeled structure and some had two, although they were not separated to the extent observed in uninduced controls at the same cell cycle stage (compare Fig. 4, A and B, with Fig. 4C). The precise nature of the inhibition remains unclear, although it clearly led to an inhibition of kinetoplast division.

Duplication of the Golgi was dependent on the presence of TbCentrin2 but not TbCentrin1. Depletion of TbCentrin2 led to the presence of only one Golgi in ~72% of the cells, whereas depletion of TbCentrin1 led to the opposite result: About 77% of the cells had two Golgi (Fig. 4D). Thus, TbCentrin2 is essential for the Golgi duplication process.

The simplest interpretation is that the bi-lobed structure containing TbCentrin2 helps determine the location at which the new Golgi assembles. One lobe is associated with the old Golgi, the other with the new. When TbCentrin2 is absent, only one Golgi is seen. What is not yet clear is whether the Golgi has doubled in mass but not separated, or whether the actual doubling process has also been inhibited. TbCentrin2 may be involved in either or both of these processes.

The location of the endoplasmic reticulum (ER) exit sites was also determined in relation to this bi-lobed structure. These sites are adjacent to the cis face of the Golgi stack in *T. brucei* and a new ER exit site grows at the same time as the new Golgi (*4*). The coat protein II (COPII) coat component, TbSec13p, a component of ER-to-Golgi transport vesicles, was closer to the cis Golgi than the TbCentrin2-containing structure at all stages of the cell cycle (fig. S2A). Because the COPII coat is present on vesicles near to the Golgi as well as on those budding from the ER exit sites, this would place at least part of the bi-lobed structure over the ER exit sites. This was confirmed at the electron microscopic level by immunolabeling thin sections from cells expressing YFP-tagged TbCentrin2. Labeling was found over basal bodies and ER exit sites but not over other parts of the ER (fig. S2B). The density of labeling over the ER exit sites was ~3 times as high as that over the Golgi stack and ~6.6 times as high as that over mitochondria (fig. S2C). Comparable experiments with cells expressing TbCentrin1 showed levels of labeling over ER exit sites only slightly higher than those over the Golgi stack and mitochondria.

Thus, Centrin2 in *T. brucei* is additionally present in a bi-lobed structure and is needed for Golgi duplication. The morphology and location of this structure suggest that it helps define the site at which a new ER exit site and Golgi are constructed. Such an arrangement might help to ensure accurate duplication and ultimate partitioning to each daughter cell. Whether this also pertains in higher eukaryotes is the focus of present research.

References and Notes

1. S. Munro, *Nat. Cell Biol.* **4**, E223 (2002).
2. B. J. Bevis, A. T. Hammond, C. A. Reinke, B. S. Glick, *Nat. Cell Biol.* **4**, 750 (2002).
3. W. G. Whaley, *The Golgi Apparatus, Cell Biology Monographs, Continuation of Protoplasmatologia 2.* (Springer-Verlag, Wien, New York, 1975).
4. C. Y. He *et al.*, *J. Cell Biol.* **165**, 313 (2004).
5. H. Field, T. Sherwin, A. C. Smith, K. Gull, M. C. Field, *Mol. Biochem. Parasitol.* **106**, 21 (2000).
6. D. R. Robinson, K. Gull, *Nature* **352**, 731 (1991).
7. B. Striepen *et al.*, *J. Cell Biol.* **151**, 1423 (2000).
8. J. L. Salisbury, *Curr. Opin. Cell Biol.* **7**, 39 (1995).
9. M. A. Sanders, J. L. Salisbury, *J. Cell Biol.* **124**, 795 (1994).
10. Materials and methods are available as supporting material on *Science* Online.
11. P. Bastin, Z. Bagherzadeh, K. R. Matthews, K. Gull, *Mol. Biochem. Parasitol.* **77**, 235 (1996).
12. M. J. Chapman, M. F. Dolan, L. Margulis, *Q. Rev. Biol.* **75**, 409 (2000).
13. R. M. Rios, A. Sanchis, A. M. Tassin, C. Fedriani, M. Bornens, *Cell* **118**, 323 (2004).
14. V. Scott, T. Sherwin, K. Gull, *J. Cell Sci.* **110**, 157 (1997).
15. T. Sherwin, A. Schneider, R. Sasse, T. Seebeck, K. Gull, *J. Cell Biol.* **104**, 439 (1987).
16. A. Djikeng, S. Shen, C. Tschudi, E. Ullu, *Methods Mol. Biol.* **270**, 287 (2004).
17. A. Paoletti *et al.*, *Mol. Biol. Cell* **14**, 2793 (2003).
18. J. L. Salisbury, K. M. Suino, R. Busby, M. Springett, *Curr. Biol.* **12**, 1287 (2002).
19. A. Selvapandiyan *et al.*, *J. Biol. Chem.* **279**, 25703 (2004).
20. We thank E. Ullu, C. de Graffenried, and H. Ho for critical reading and helpful suggestions; J. Yelinek for help with the electron microscopy; and J. Salisbury for the gift of monoclonal 20H5 antibodies to Centrin. This work was supported by the NIH.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1119969/DC1
Materials and Methods
Figs. S1 and S2
References

9 September 2005; accepted 20 October 2005
Published online 27 October 2005;
10.1126/science.1119969
Include this information when citing this paper.

Dendritic Cell Kits

Two novel kits for the isolation of human plasmacytoid dendritic cells (PDCs) open new perspectives for dendritic cell research. The Plasmacytoid Dendritic Cell Isolation Kit makes it possible to isolate untouched PDCs by depleting non-PDCs with a cocktail of biotinylated antibodies and MACS microbeads. The isolation of untouched PDCs is particularly useful if minimal antibody binding to the cell surface antigens should be avoided for downstream applications. The Diamond Plasmacytoid Dendritic Cell Isolation Kit allows the isolation of PDCs of almost 100% purity, so offers a fast and convenient alternative to flow cytometric cell sorting.
Miltenyi Biotec. For information +49 2204 8306-37 www.miltenyibiotec.com

Direct Analysis in Real Time

The DART (direct analysis in real time) is an ion source for the Jeol AccuTOF time-of-flight mass spectrometer. DART is designed to enable direct detection of chemicals on surfaces, in liquids, and in gases without the need for sample preparation. With DART, samples are analyzed in open air, using no radioactive components, solvent sprays, exposed high voltage, or vacuum to alter the sample state. DART often eliminates sample preparation time and complements existing liquid chromatography or gas chromatography techniques.
Jeol. For information 978-536-2309 www.jeolusa.com

Low-Abundance Protein Detection

Equalizer Beads can reduce the concentration differences of proteins in biological liquids to enable detection of low-abundance proteins. The method is rapid, requires only microliters of sample, is applicable to a wide variety of biological fluids, and is amenable to automation to facilitate biomarker discovery. Although the human plasma proteome comprises thousands of different proteins and polypeptides, only a few dozen of them make up 99% of the protein mass. Considerable effort has been expended in the field of proteomics to identify these proteins and understand their compositions and functions in disease processes. Current detection methods have been insufficient to cover the concentration range of proteins in blood, which impeded the detection of proteins that may represent new diagnostic and prognostic indicators or therapeutic targets. The Equalizer process could increase the number of detectable proteins and improve the discovery of pathologically relevant biomarkers.
Ciphergen For information 888-864-3770 www.ciphergen.com

For more information visit GetInfo, *Science*'s new online product index at *http://science.labvelocity.com*

From the pages of GetInfo, you can:
- Quickly find and request free information on products and services found in the pages of *Science*.
- Ask vendors to contact you with more information.
- Link directly to vendors' Web sites.

Human Exon Array

The GeneChip Human Exon 1.0 ST Array offers whole-genome, exon-level expression profiling on a single array. Designed to interrogate about 1 million exons, the array helps researchers better understand the roles that alternative splicing and gene expression play. At least 60 percent of the best-characterized genes in the human genome comprise multiple exons, or small blocks of RNA that can be rearranged to create different transcripts from the same gene. Each of these transcripts can potentially be translated into a different protein carrying out different functions, which greatly increases the diversity of proteins generated from the genome. The GeneChip exon arrays offer a high resolution view to examine these alternatively expressed exons, enabling researchers to conduct experiments that were not possible before.
Affymetrix For information 408-731-5791 www.affymetrix.com

Heating Circulators

The 8100 Series is a line of medium-capacity heating circulators designed to provide precise temperature control for a diverse range of applications. The circulators are available with a choice of three microprocessor-based controllers, integrated simplex or duplex pump, and 13-l reservoir. The units feature temperatures as high as 150°C and excellent temperature stability. They require only about 1.1 square feet of bench space. The top-of-the-line 8112 Circulator features a programmable controller with bright LCD display, ambient +5° to 150°C temperature control, ±0.01°C temperature stability, multi-language help menus, and sophisticated time/temperature programming. It can store up to ten 50-step programs and offers extensive data logging capability, including communications support for Microsoft Excel, National Instruments LabView, and the Palm OS. Other standard features include a five-speed pump, remote probe control capability, and a built-in RS-232 interface.
PolyScience For information 800-229-7569 www.polyscience.com

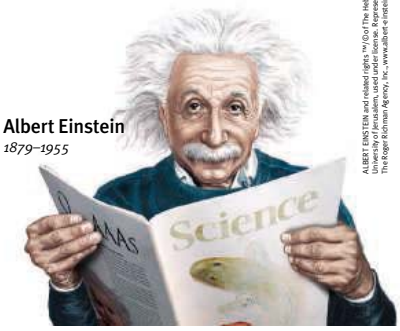
Dual Access Flow Cabinet

The PuriCare Dual Access Laminar Flow Cabinet is designed for permanent installation through an opening in the wall between a vivarium "clean" room and a "dirty" room that is exposed to dust and other particles present in the lab environment. The cabinet provides Class 100 clean air in the work area. Personnel on one side can load mice, cages, food, medications, and other supplies through the sash opening into the cabinet while the sash on the opposite side remains closed. High-efficiency particulate air (HEPA) filtered air purges the contents of the cabinet during the transfer and when both sashes are closed. After allowing the HEPA-filtered laminar air to circulate inside the cabinet, cleanroom personnel can then open the opposite-side sash and retrieve the supplies without exposing themselves or the cleanroom to airborne contaminants. Features include audible/visual sash alarms, two interior-mounted electrical duplexes, and two pressure gauges for monitoring differential pressure across the upper and lower HEPA filters.
Labconco For information 800-821-5525 www.labconco.com

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and government organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier by visiting www.science.labvelocity.com on the Web, where you can request that the information be sent to you by e-mail, fax, mail, or telephone.

ScienceCareers.org

Classified Advertising



Albert Einstein
1879–1955

For full advertising details, go to **www.sciencecareers.org** and click on **How to Advertise,** or call one of our representatives.

United States & Canada

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

JILL DOWNING
(CT, DE, DC, FL, GA, MD, ME, MA, NH, NJ, NY, NC, PA, RI, SC, VT, VA)
Phone: 631-580-2445

KRISTINE VON ZEDLITZ
(AK, AZ, CA, CO, HI, ID, IA, KS, MT, NE, NV, NM, ND, OR, SD, TX, UT, WA, WY)
Phone: 415-956-2531

KATHLEEN CLARK
Employment: AR, IL, LA, MN, MO, OK, WI, Canada; Graduate Programs; Meetings & Announcements (U.S., Canada, Caribbean, Central and South America)
Phone: 510-271-8349

EMNET TESFAYE
(Display Ads: AL, IN, KY, MI, MS, OH, TN, WV; Line Ads)
Phone: 202-326-6740

GABRIELLE BOGUSLAWSKI
(U.S. Recruitment Advertising Sales Director)
Phone: 718-491-1607

Europe & International

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223-326-532

TRACY HOLMES
Phone: +44 (0) 1223-326-525

HELEN MORONEY
Phone: +44 (0) 1223-326-528

CHRISTINA HARRISON
Phone: +44 (0) 1223-326-510

SVITLANA BARNES
Phone: +44 (0) 1223-326-527

JASON HANNAFORD
Phone: +81 (0) 52-789-1860

To subscribe to *Science*:
In U.S./Canada call 202-326-6417 or 1-800-731-4939
In the rest of the world call +44 (0) 1223-326-515

Science makes every effort to screen its ads for offensive and/or discriminatory language in accordance with U.S. and non-U.S. law. Since we are an international journal, you may see ads from non-U.S. countries that request applications from specific demographic groups. Since U.S. law does not apply to other countries we try to accommodate recruiting practices of other countries. However, we encourage our readers to alert us to any ads that they feel are discriminatory or offensive.

POSITIONS OPEN

GEOLOGY/ENVIRONMENTAL SCIENTIST TENURE-TRACK FACULTY POSITION

The Department of Biological, Geological and Environmental Sciences at Cleveland State University (CSU) invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level with expertise in hydrogeology or related field. We particularly encourage applicants with a research emphasis in one or more of the following areas: watershed-scale processes, environmental applications of hydrogeology, linkages between geochemical and ecological processes, applied geographic information systems, water resource management and/or restoration. This position will complement existing research within our Department on land and water resources and urban environmental issues involving collaborative partnerships with a number of regional universities, museums, county, state and national parks, and local and state agencies. Qualified applicants must have a Ph.D. and two years of post-doctoral or equivalent professional experience and must demonstrate the potential for excellence in research and teaching in geology and environmental science. Candidates with teaching experience, a record of publication in appropriate peer-reviewed journals, potential for obtaining external funding, and potential for contributing to the doctoral program are preferred. The successful candidate will be expected (1) to establish an independent, externally funded research program, (2) to supervise undergraduate and graduate research, and (3) to have a commitment to undergraduate and graduate teaching. Competitive salary and startup funds. Starting date is August 21, 2006.

Before January 2, 2006, applicants must submit curriculum vitae, a description of research plans and teaching interests, up to three reprints, and three letters of recommendation to be sent to: **Dr. Julie A. Wolin, Chair, Faculty Search Committee, Department of Biological, Geological and Environmental Sciences, Cleveland State University, 2121 Euclid Avenue, Cleveland OH 44115-2214.** Applications will also be accepted as PDF attachments to **e-mail: j.wolin@csuohio.edu.** Letters of recommendation, however, must be sent by mail.

For additional information, consult the Department's **website: http://web.bges.csuohio.edu** and the Cleveland State Environmental Institute **website: http://www.csuohio.edu/ei/index.html** or contact: **Dr. Wolin, e-mail: j.wolin@csuohio.edu; telephone: 216-687-3505.** *CSU is an Affirmative Action/Equal Opportunity Employer Institution committed to nondiscrimination in employment and education. Minorities/Females/Persons with Disabilities/Veterans encouraged.*

Biology, ASSISTANT PROFESSOR. Mount Mercy College announces an opening for a tenure-track position to begin August 2006. We seek a broadly trained Biologist with expertise in developing a course in molecular biology. Primary responsibilities include teaching an upper-level genetics course to biology majors as well as separate courses in human anatomy and human physiology lectures with anatomy laboratories to a mixture of nursing and biology majors and physiology laboratories for biology majors. Experience with human cadavers is desirable. The teaching load may also include a course in nonmajor human biology. Candidates must have a Ph.D.; prior teaching experience is strongly preferred. Applications should be sent to: **Dr. Neil Bernstein, Department of Biology, Mount Mercy College, 1330 Elmhurst Drive N.E., Cedar Rapids, IA 52402.** Include a complete curriculum vitae, copies of transcripts, a statement of teaching philosophy that addresses the role of science in a liberal arts/professional curriculum, a statement that documents your ability to teach the above courses, a statement of what your professional goals would be in a small, undergraduate college, and contact information for three references. Copies of teaching evaluations are desirable. Applications must be received by December 30, 2005. **Website: http://www2.mtmercy.edu/.** *Equal Opportunity Employer committed to diversity.*

POSITIONS OPEN

FACULTY POSITION
Department of Biochemistry and Molecular Biology
The University of North Dakota
School of Medicine and Health Sciences

The Department of Biochemistry and Molecular Biology of the University of North Dakota School of Medicine and Health Sciences invites applications for a tenure-track position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level, dependent on qualifications. The 12-month, state-supported position begins August 2006. The successful candidate must have a strong potential for NIH funding. The candidate's research program should complement existing departmental research interests. The candidate will participate in a nationally recognized patient-centered-learning medical school curriculum as well as teach in a graduate program that offers M.S., Ph.D., and M.D./Ph.D. degrees. The Department currently has 10 faculty members with research interests in regulation of dopamine transporter and cocaine addiction and related drug addiction, drug resistance mechanisms in human pathogens, calcium signaling, bacteria-host interactions and inflammation, signaling in retinal degeneration, matrix signaling pathways in osteoarthritis, gene regulation and cancer, and toxicology. The successful candidate will enjoy access to a modern animal facility, computational chemistry and biology network, light, electron and confocal fluorescence microscopy facility, and proteomics/mass spectrometry core facility. The University of approximately 14,000 students is located in Grand Forks, a family-friendly community with excellent public schools and many cultural, recreational, and sporting activities. Further information on the position can be found at **website: http://www.med.und.nodak.edu/bimd/biochem.html** and on the local environment at **websites: http://www.und.nodak.edu** and **http://www.grandforksgov.com.** Applicants should submit a cover letter detailing their future research/career plans, curriculum vitae, and the addresses of three references to: **Search Committee, Department of Biochemistry and Molecular Biology, University of North Dakota School of Medicine and Health Sciences, P.O. Box 9037, Grand Forks, ND 58202.** Review of applications will begin December 12, 2005, and the search will remain open until the position is filled. *The University of North Dakota is an Equal Opportunity/Affirmative Action Institution.*

ASSISTANT PROFESSOR
BIOMEDICAL SCIENCES
The University of Akron

Department of Biology invites applications for a tenure-track position in the area of biomedical sciences at the rank of Assistant Professor, to begin August 28, 2006. Applicants with research and teaching experience in functional genomics, physiology, or virology are encouraged to apply. We are especially interested in applicants who will contribute to our program in Integrative Biosciences. Candidates must hold a Ph.D. degree, and post-doctoral experience is preferred. Successful candidates will be expected to develop an externally funded research program in their area of expertise, advise graduate students, and contribute to the undergraduate and graduate biology curricula. The University of Akron is one of the largest state universities in Ohio with over 24,000 students. For further information visit the Department's **website: http://www.uakron.edu/biology/.** Review of applications will begin December 9, 2005. Applicants should submit curriculum vitae, a summary of teaching interests, a research statement, copies of recent publications, and three letters of recommendation to: **Chair, Biomedical Search Committee, Department of Biology, The University of Akron, Akron, OH 44325-3908.** *The University of Akron is committed to a policy of Equal Employment Opportunity and to the principles of Affirmative Action in accordance with state and federal laws.*

PENNSTATE
University Park

Director
Dorothy Foehr Huck and J. Lloyd Huck Institutes of the Life Sciences

The Search Committee for the Director of the Huck Institutes of the Life Sciences seeks nominations and applications for an innovative Director of the Huck Institutes of the Life Sciences. The Huck Institutes is a novel university-wide organization whose mission is to strengthen research and education in the life/health sciences at Penn State through inter- and cross disciplinary approaches. Penn State is committed to building academic and research excellence in the life sciences. The new Director has the opportunity to build upon the exciting advances in the life sciences at Penn State over the past decade and play a prominent role in the strategic directions of this endeavor.

The Huck Institutes – including the Institute for Genomics, Proteomics, and Bioinformatics, the Biotechnology Institute, and the Neuroscience Institute – comprise a virtual organization within seven of Penn State's colleges, including the College of Medicine at Hershey. The purpose of the Huck Institutes is to enhance Penn State's ability to prepare students for tomorrow's challenges and to support the integration of research and teaching across disciplines in the life sciences at Penn State. Core funding for the Huck Institutes is provided by the University.

As part of the Huck Institutes initiative, the University has committed the funds to recruit new faculty into key disciplines. Also supported are twelve state-of-the-art shared technology facilities, including the recently established MRI for laboratory animals, proteomics and mass spectrometry and macromolecular X-ray crystallography facilities (http://www.huck.psu.edu/stf/home.html). Further support comes from the State's Tobacco Settlement Funds, which offer a unique opportunity to initiate creative life sciences programs. Cohesion and growth of these interdisciplinary initiatives is enabled, in part, by the facilities in the 145,000-square-foot Life Sciences Building (completed in the fall of 2004), by recent renovations to the adjacent Wartik Building to house the Institute for Genomics, Proteomics and Bioinformatics, and another contiguous life science building to be completed in 2008. The Huck Institutes often engage other interdisciplinary institutes at Penn State, including the Penn State Institutes of the Environment, Materials Research Institute, and the Social Sciences Research Institute. Another exciting initiative is a planned new building for the Materials Research Institute that will physically couple with Penn State's planned new Life Sciences Building; nanobiotechnology, biomaterials, and biosensor activities are envisioned in this enriched setting.

The Director of the Huck Institutes reports directly to the Vice President for Research, who oversees a research activity exceeding $635 million annually. The responsibilities of the Director include: (1) supporting research and education at the interface between the life sciences, technology, and business; (2) proactively fostering collaboration among the core colleges and developing policies and incentives for active faculty participation, which build an atmosphere of inclusion of diverse perspectives; (3) chairing the faculty steering committee which oversees all activities and is responsible for developing policies and programs for the Huck Institutes; (4) chairing the Dean's Advisory Committee, which gives advice to the Director on operational, financial, and space matters; (5) identifying exciting new funding opportunities and coordinating interdisciplinary research programs; (6) enhancing graduate and undergraduate education in the life sciences including supporting graduate students through fellowships, assistantships, joint recruiting, and counseling; (7) supporting a variety of enhancements including seminars, workshops, and symposia; and (8) serving as liaison between the University's life sciences programs and state, national, and international life sciences organizations.

The successful candidate will be an international leader in the life sciences, recognized for scientific accomplishment and vision and the skills necessary to develop new initiatives and advance ongoing programs in research, teaching, and outreach in the life sciences within the academic environment of this land grant university. Candidates must have qualifications suitable for a tenured faculty appointment in one of the University's academic colleges. Review of applications will begin on December 14, 2005, and will continue until the position is filled. Nominations may be sent via e-mail to vxi2@psu.edu. Applicants should send a letter with a detailed visionary statement, a resume or curriculum vitae, and the names, addresses, telephone numbers, and e-mail addresses of four references via an e-mail to:

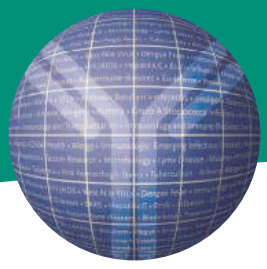
William Easterling, Chair
Search Committee for Director of the Huck Institutes
The Pennsylvania State University
304 Old Main
University Park, PA 16802
E-mail: vxi2@psu.edu

Interested individuals are invited to visit the following web site for more information: www.huck.psu.edu

Penn State is committed to affirmative action, equal opportunity and the diversity of its workforce.

PENN STATE ***Making Life Better***

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH



Health Research in a Changing World

Fighting Diseases and Improving Lives

Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases

Tenure Track Position in Immunology

The Laboratory of Immunology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health invites applications for a tenure track position in immunology. While applicants in any area of immunological science will be considered, those interested in the immunology of infectious diseases, in B-cell biology or in innate immunity are particularly encouraged to apply. Applicants should have a Ph.D., M.D. or equivalent degree and a strong record of post-doctoral scientific accomplishment. The successful candidate will be expected to establish an independent research program. Support for salary, technical personnel, post-doctoral fellows, equipment and research supplies will be provided. Current PIs in the Laboratory of Immunology are: Ronald Germain, Michael Lenardo, Rose Mage, David Margulies, William Paul, and Ethan Shevach. Active areas of research include lymphocyte development and dynamics; B-cell development, selection and diversification processes; MHC molecule, NK cell receptor and T cell receptor structure and function; dendritic cells and antigen processing; T cell and cytokine receptor signal transduction; cell death in normal immune responses and in HIV infection; regulation of cytokine expression and activity of cytokines; regulatory T cells and control of autoimmune responses; advanced imaging of immune cell interactions; and mathematical modeling of signal transduction in immune cells.

Applicants should send a CV and bibliography, outline of a proposed research program (no more than two pages), and three letters of recommendation to Dr. Jonathan Ashwell, Chair, NIAID Search Committee, C/O Mrs. Lynn Novelli, Committee Manager, 10 Center Drive MSC 1356, Building 10, Room 4A26, Bethesda, Maryland 20892-1356. Applications must be received by **December 15, 2005**.

A full package of benefits (including retirement, health, life and long term care insurance, Thrift Savings Plan participation, etc.) is available.

We invite to you explore our Institute and other opportunities at http://healthresearch.niaid.nih.gov/science.

Please reference "Science" on your resume

NIEHS National Institute of Environmental Health Sciences

Tenure-Track Investigator
Cellular and Molecular Biologist
Research Triangle Park, North Carolina

With nation-wide responsibility for improving the health and well being of all Americans, The Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health.The National Institute of Environmental Health Sciences, a major research component of the National Institutes of Health (NIH) and the Department of Health and Human (HHS) is recruiting a Tenure -Track Investigator-Cellular and Molecular Biologist with research strengths in metabolic nuclear receptors such as peroxisome proliferator-activated receptors (PPARS) and their fundamental mechanisms of action in relation to diseases such as obesity. Research expertise in signaling, transcriptional/translational regulatory mechanisms and molecular imaging is desirable. Model systems need not be restricted to mammals. Applicants should have a Ph.D., MD/Ph.D., MD or equivalent with 3 years of postdoctoral research experience and a strong publication record. The successful applicant will be expected to establish a high-quality independent research program in the area of PPARS or other metabolic nuclear receptors within a basic science research Branch having diverse interests in molecular signaling mechanisms. The applicant will have open access to state-of-the-art equipment and outstanding core facilities (imaging, flowcytometry, microarrays, mass spectrometry, mouse genetics, protein expression, and crystalography). Opportunities exist for interactions with expanding clinical research programs. Excellent laboratory space, start up funds, salary and benefits will be provided. The time before tenure review will be dependent on qualifications but will not exceed 6 years. For additional information about this position, contact **Dr. John A. Cidlowski, Chief, Laboratory of Signal Transduction (cidlows1@niehs.nih.gov).** Highly qualified applicants should send their curriculum vita, with a one page statement of research plan and arrange for three letters of recommendation to be sent to the following address by **December 31, 2005.** Applications received after **December 31, 2005,** will be considered as needed until the position is filled. **Ms. Lisa Rogers (DIR05-11), National Institutes of Health, National Institute of Environmental Health Sciences, P.O. Box 12233, Maildrop A2-06, 111 Alexander Drive, Room A208, Research Triangle Park, NC 27709, e-mail: dir-appls@niehs.nih.gov**

Health Scientist Administrator

The National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Department of Health & Human Services (DHHS) is seeking applicants for a Health Scientist Administrator position in the Center for Biotechnology and Innovation (CBI). The position is for a Director of the Applied and Translational Research Program. This program emphasizes interdisciplinary/multidisciplinary, highly innovative approach that combines engineering, physics, biology and clinical dental medicine for the restoration/regeneration of orofacial structures (e.g., teeth, bone, salivary glands, periodontal and temporomandibular joint structures, etc.) Relevant areas include: studies regarding the design of bio-inspired new dental composite/ceramic materials through biomimetic principles, a systems approach to the design and development of new biocompatible/inductive materials that can stimulate cells and tissues to regenerate and/or materials that can become integrated into the body, use of stem cells and biomimetic approaches in regenerating soft and hard tissues structures of the craniofacial region, computational methods for multiple scaffold designs that can promote stem cell assembly into multi-dimensional structures, design and development of integrated microfluidic platforms based on multiple separation and detection technologies on a single chip in order to obtain inexpensive, rapid detection technologies for biological processes in health and disease, development of delivery vehicles (nanoparticles, artificial matrices) and development of micro-environments where cells can be precisely placed, manipulated and then analyzed in real time. The incumbent will direct, administer and evaluate a portfolio of extramural grants, contracts and cooperative agreements and will stimulate interest in and provide advice to the extramural community regarding the respective portfolio. In addition, the incumbent will participate in funding decisions, policy development, as well as implementation and coordination with other programs both within and outside of the NIDCR. The applicant is required to have a D.D.S., D.M.D., M.D., Ph.D. (or equivalent doctoral degree). The salary range for this position is $88,369 to $114,882 per annum, commensurate with experience. This position has knowledges, skills and abilities (KSA) that must be addressed in order for applicants to be considered. The full vacancy announcement can be viewed at www.usa.jobs under NIDCR-05-98604. Applications will be accepted until **November 21, 2005**. Please submit materials to: **Elan Ey, Branch I, Office of Human Resources, NIH, 6707 Democracy Blvd., Suite 400, Bethesda, MD 20892-5482 or by email: elan.ey@nih.gov.** U.S. Citizenship is required.

WWW.NIH.GOV

National Institute of General Medical Sciences
National Institutes of Health

The National Institute of General Medical Sciences (NIGMS) in Bethesda, MD is seeking applications from outstanding candidates for a Health Scientist Administrator (HSA) position in the Pharmacological and Physiological Sciences Branch within the Pharmacology, Physiology, and Biological Chemistry Division. The recruiting branch currently supports research and training into understanding the basis of traumatic and burn injury and the perioperative period, the molecular basis of action of anesthetics, the mechanisms of and genetics underlying the actions of therapeutic drugs, and the development of predictive preclinical toxicology approaches.

The individual hired will be responsible for applying his/her clinical and research expertise to manage and develop research and training grants in NIGMS' broad areas of basic studies in pharmacological and physiological sciences, and to foster the translation of results from fundamental research areas into clinical studies. The person should have experience gained in a medical research institution and understand how research is conducted with human subjects or patients in a clinical setting. A background in at least one of the following areas is preferred: trauma, injury and recovery, or clinical pharmacology, or immune system biology, or alternatively in a cross-cutting area such as studies of the role of inflammation in the disease process or of molecular/cellular signaling in these systems. Experience in modern methods of genome or proteome analysis would also be desirable.

Applicants must possess an MD and/or PhD plus scientific knowledge in the fields of pharmacology, physiology, immunology, systems biology, medicine, or related fields. Applicants must be familiar with both clinical and laboratory approaches in his/her own field(s) of expertise. Experience in the NIH peer review and grant award process would be beneficial. Salary will be commensurate with qualifications, may include a physician's comparability allowance, and will have a full package of benefits. Detailed vacancy announcements **NIGMS-05-100271** and **NIGMS-05-100881** with the qualifications and application procedures are available at the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html**. Questions about application procedures may be directed to **Erin Bandak at 301-594-2324**. Applications must be received by **January 4, 2006**.

Health Scientist Administrator
National Institute of General Medical Sciences

The National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland is seeking applications from outstanding candidates for one Health Scientist Administrator position in the Division of Pharmacology, Physiology and Biological Chemistry, which supports primarily basic, non-disease-oriented research and training, including a substantial portfolio of bio-related chemical research.

The incumbent for this position will be responsible for developing and managing a portfolio of research grants that support studies that utilize organic chemistry to understand and/or control biological systems. The ideal candidate will have a substantial background in the design, synthesis and evaluation of small organic molecules as well as a thorough grounding in such areas as biochemistry, pharmacology or molecular biology. Prior research experience in bio-related organic chemistry is desirable.

Applicants must possess a Ph.D. or M.D. plus scientific knowledge and demonstrated expertise in at least one of the following areas: organic chemistry, biochemistry, pharmacology, or related areas, and knowledge of the NIH peer review and grants process. Salary is commensurate with qualifications, and includes a full package of benefits. A detailed vacancy announcement **(NIGMS-05-100284)** with the mandatory qualifications and application procedures can be obtained via the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html** and NIH Home page at **http://www.jobs.nih.gov**. Questions on application procedures may be addressed to **Erin Bandak at (301) 594-2324**. Applications must be received by close of business **January 6, 2006**.

Health Scientist Administrator
National Institute of General Medical Sciences

The National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland is seeking applications from outstanding candidates for a Health Scientist Administrator position in the Minority Biomedical Research Support (MBRS) Branch of the Minority Opportunities in Research (MORE) Division.

The incumbent for this position will be responsible for the management of a portfolio of grants from the pre-application phase through advisory council preparation and follow-up. The incumbent will provide scientific expertise in specialized areas of the disciplines relevant to biomedical and/or behavioral research, and will advise grantees and professional staff members regarding aspects of grants within the areas of expertise. The incumbent will also make recommendations on the funding of specific applications, attend meetings of study sections, review committees, and advisory council, and participate in site visits. Additionally, the incumbent will manage the post-award phase of a portfolio of grants, including monitoring and evaluating grant outcomes, and prepare reports of scientific progress.

Applicants must possess a Ph.D. or equivalent degree plus scientific knowledge and demonstrated expertise in at least one of the following areas: biochemistry, microbiology, organic chemistry, physiology, genetics, psychology, or related areas. Salary is commensurate with qualifications, and includes a full package of benefits. A detailed vacancy announcement with the mandatory qualifications and application procedures can be obtained via the NIGMS web site at **http://www.nigms.nih.gov/About/Job_Vacancies.htm** and the NIH website at **http://www.jobs.nih.gov**. Questions on application procedures may be addressed to **Erin Bandak at (301) 594-2324.** Applications must be received by close of business **December 20, 2005.**

DEAN
USC LEONARD DAVIS SCHOOL OF GERONTOLOGY

The University of Southern California (USC) invites applications and nominations for the position of Dean of the Leonard Davis School of Gerontology.

USC founded in 1880, is a major private research-intensive university located in a multicultural urban setting. USC is comprised of 19 colleges including health professional schools of pharmacy, dentistry, gerontology, medicine and the allied health sciences, and is one of the largest private universities in the United States with 4,200 full-time faculty and 30,000 students.

The Leonard Davis School of Gerontology is an international leader in the field of gerontology. Founded in 1975, the Leonard Davis School faculty represents disciplines important to the study of aging. These include policy, sociology, psychology, neurobiology and molecular biology, demography, and medicine. Research programs are highly interdisciplinary, and include major projects on Alzheimer disease, fall prevention, cognitive loss, family relationships, bio-demography, and the evolution of longevity. The Davis School has educational programs at all levels: a Ph.D. in Gerontology, professionally oriented Master's degree, undergraduate degrees oriented toward the health and professional tracks. Two NIH Training grants support pre- and postdoctoral students across USC. The Davis School is also engaged in outreach and information dissemination projects with both public and private organizations.

The new Dean must possess an exceptional academic and/or professional reputation, demonstrated executive skills, creativity, and be strongly motivated to expand the educational and research accomplishments in a highly visible environment. The Dean must energetically promote collaboration in research and education, including building partnerships with USC's College of Letters, Arts and Sciences; Viterbi School of Engineering, School of Dentistry and Keck School of Medicine. Excellent communication and interpersonal abilities are essential. The Dean will work closely with and further develop relationships with faculty, students, parents, alumni, and donors.

Credentials will be reviewed during November/December 2005. Letters of nomination and application, including curriculum vitae and three professional references, may be mailed to: **Harold C. Slavkin, Chair, Dean of Gerontology Search Committee, Dean, School of Dentistry, University of Southern California, 925 West 34th Street, DEN 203, Los Angeles, CA 90089-0641.** For more information about the Davis School of Gerontology, please visit **www.usc.edu/dept/gero/**.

USC is an Equal Opportunity/Affirmative Action employer. Minority and female applicants are encouraged to apply.

Faculty Opening
Drug/Gene Delivery and Targeting

The Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina at Chapel Hill, is seeking to fill a full time, tenure-track position at any rank. Successful candidates must possess a Ph.D. degree, or equivalent, and appropriate postdoctoral experience in the broad area of drug/gene delivery and targeting. The person is expected to participate in teaching at both professional and graduate levels and to establish an outstanding research program that is fundable or already funded by NIH. Please visit the School's website at **www.pharmacy.unc.edu**.

Women and members of minority groups are encouraged to apply. Applications, including a CV, statement of research interest, and the names and contact information of three references should be submitted to:

Leaf Huang, Ph.D.
Chair, Division of
Molecular Pharmaceutics
School of Pharmacy
2316 Kerr Hall, CB# 7360
University of North Carolina
Chapel Hill, NC 27599-7360
Or by email to: **leafh@unc.edu**

The University of North Carolina is an Equal Opportunity/Affirmative Action Employer.

VIROLOGY, CANCER VIROLOGY FACULTY POSITIONS

Department of Molecular Biology and Microbiology
Case Western Reserve University
School of Medicine

The Department of Molecular Biology and Microbiology at CWRU School of Medicine is undergoing significant expansion under our new Chair, **Dr. Jonathan Karn**. At least **four faculty recruitments** are planned over the next few years, with positions available at the **Assistant/Associate/Professor** level. We encourage applications from highly qualified individuals with demonstrated experience in the areas of:

- **Molecular Virology (MV).** Fundamental aspects of Virology (e.g. molecular mechanisms in viral replication; virus assembly and maturation; control of latency; host cell interactions; virus entry mechanisms; viral diversity and immune evasion).
- **Cancer Virology (CV).** Basic virology leading to a fundamental understanding of the molecular basis for tumorigenesis. This includes interests in the discovery of new human pathogens, viral etiology of cancer, viral models for cancer.

Successful candidates will establish a vigorous research program, participate in teaching activities, and interact productively with basic and clinical scientists interested in microbiology and infectious diseases. In addition to newly refurbished laboratory space and generous start-up packages, we offer a highly interactive environment with exceptional intellectual, infrastructural, and administrative support. Further details are available at: **http://sunshine.case.edu/mvircvirapp/**.

Please submit a letter of application, curriculum vitae, brief statement of research goals and accomplishments, and 3 references to: **Jonathan Karn, Ph.D., Chair; Virology Search Committee** online at the website above. Questions may be directed to Mrs. Omabegho at **beo3@case.edu**.

Evaluation of the applications will commence in **January 2006**.

In employment as in education Case Western Reserve University is committed to EOE/AA.

THE CLEVELAND CLINIC

CHAIR
DEPARTMENT OF CANCER BIOLOGY
LERNER RESEARCH INSTITUTE
THE CLEVELAND CLINIC FOUNDATION

The Cleveland Clinic Foundation is seeking a Chair for the Department of Cancer Biology, which occupies ~30,000 sq. ft. in the Lerner Research Institute. An endowed chair accompanies this position. The department currently consists of 12 faculty with well-funded research programs in the areas of signal transduction and gene expression, apoptosis, cytokine action, and cell cycle regulation. The ideal applicant will have an outstanding national reputation in an area that complements the strengths of the department. The Chair of Cancer Biology will also hold appointment as Associate Director for Basic Science of the Cleveland Clinic Taussig Cancer Center, and will have a cross appointment in the matrix format of the NCI-designated Case Comprehensive Cancer Center.

The Chair will be provided with generous start-up support and recruitment packages for 4 to 5 new faculty. The Lerner Research Institute with over 130 independent investigators in 10 departments and an annual budget of >$110 million has a commitment to excellence in basic and applied biomedical research.

A curriculum vitae and letter of interest should be sent to:

Paul E. DiCorleto, Ph.D.
Search Committee for the Chair of Cancer Biology
Cleveland Clinic Lerner Research Institute – NB21
9500 Euclid Avenue
Cleveland Ohio 44195

On the Web - **http://www.lerner.ccf.org/cancerbio/**
E-mail: **dicorlp@ccf.org**

The Cleveland Clinic Foundation is an Equal Opportunity Employer.

US Department of Energy
Office of Science
Office of Basic Energy Sciences

Program Manager for Chemical Physics

Chemical Sciences, Geosciences and Biosciences Division
Announcement Number PN-05-SC22-006

The U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences (BES), Chemical Sciences, Geosciences and Biosciences Division is seeking applicants for a Chemist, GS-1320-15, with a CY2005 salary range of $103,947 to $135,136 per annum. The incumbent will serve as a program manager within the Chemical Physics program. The incumbent conceives, justifies, plans, initiates, manages, and coordinates aspects of this program consisting of a broad range of theoretical and experimental research aimed at a molecular level understanding of physical and chemical processes in the condensed phase and at interfaces (gas-liquid, gas-solid and liquid-solid). This research includes, but is not limited to, fundamental studies of the structure and dynamics of water and aqueous solutions, chemical reaction dynamics on surfaces and at interfaces relevant to heterogeneous catalysis and the transport of environmental wastes, and the physical and chemical properties of nano-structured materials, including clusters. The incumbent will act as a recognized expert in condensed phase and interfacial molecular sciences and will assist with new program planning and current program conduct in other core research areas within the Division related to chemical physics, including atomic, molecular and optical science, photophysics, biophysics and radiation sciences. The incumbent will also develop connections between the projects supported within the chemical physics program and the other research programs in the Division and throughout BES, including new opportunities and challenges in complex physical systems, nanoscale materials, chemical imaging, and heterogeneous catalysis. Potential applicants can learn more about the Chemical Physics program and related programs in Photochemistry and Radiation Research and Atomic, Molecular and Optical Sciences by visiting **http://www.sc.doe.gov/bes/chm/chmhome.html**.

For further information about this position and the instructions on how to apply and submit an application, please go the following website: **https://jobsonline.doe.gov/**. To be considered for this position you must apply online. Faxed or email applications will NOT be accepted. It is imperative that you follow the instructions as stated on the announcement (**PN-05-SC22-006**) located at the website indicated above for DOE JOBS.

The Department of Energy is an Equal Opportunity Employer. All qualified applicants will be considered without regard to race, religion, color, sex, age, national origin, lawful political affiliation, marital status, union membership, or other non-qualifying physical or mental handicaps.

Tenure-Track Faculty Position
NEUROSCIENCE / NEURO-ONCOLOGY

The Department of Neurosurgery at SUNY Upstate Medical University invites applications for a tenure-track faculty position at any level, beginning in the fall of 2006. We seek an outstanding scientist to lead the department's research effort in neuro-oncology. Preference will be given to applicants with expertise in signaling mechanisms controlling cell growth, differentiation, apoptosis and/or angiogenesis in cancer. Candidates must have a PhD, MD or equivalent, relevant postdoctoral experience and a strong record of research accomplishments. The research environment would include interactions with other faculty, clinicians, residents, medical students and graduate students in the neurosciences and allied fields.

The successful candidate will be expected to establish and maintain a vigorous and independently funded research program. Competitive salary and startup packages will be provided.

Applicants should submit a cover letter, curriculum vitae including a publications list and funding list, statement of research interests and three letters of reference. Review of applications will begin December 1, 2005 and continue until the position is filled. Application materials should be sent to: **Research Faculty Search Committee, c/o Norma Horton, Dept of Neurosurgery, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210.**

State University of New York
Upstate Medical University
Formerly known as SUNY Health Science Center

An AA/EEO/ADA employer, committed to excellence through diversity.

Mekong River Commission

The role of MRC is to co-ordinate and promote co-operation in all fields of sustainable development, utilisation, management and conservation of the water and related resources of the Mekong Basin.
MRC is looking for dynamic and qualified international candidates to fill the vacancy of:

Fisheries Component Coordinator
(Fisheries Ecology, Valuation and Mitigation)

This position is based in Phnom Penh, Cambodia for a duration of approximately 4 years. The post level is L-5 (similar to the P-5 post level of the UN System).

Job Summary: The Fisheries Component Coordinator has particular responsibility for activities related to Fisheries Ecology, Valuation and Mitigation component. The area of operation is in the Mekong region of Cambodia, Lao PDR, Thailand and Viet Nam. Implementation of the work programme is primarily through the national fisheries agencies of these countries.

Selection Criteria:

- M.Sc or Ph.D. in a fisheries related discipline;

Extensive experience in fisheries management, research and development, preferably in river systems;

- Extensive experience in leading research and development projects (demonstrable planning skills), including team supervision and coordination;
- Extensive experience working in developing countries, preferably in MRC member countries;
- Demonstrated excellence in writing, presenting, and reporting skills.

Application procedures: Applications must include a 2-4 page Cover Letter outlining clearly how the candidate meets the above-mentioned selection criteria. In addition to the letter, applicants should include a copy of their detailed CV and copies of at least 2 references together with a passport-size photo and contact address to: **Mekong River Commission Secretariat, P.O. Box 6101, Vientiane 01000, Lao PDR. Email: mrcs@mrcmekong.org**
Closing date for application: 14 December 2005.
Women are encouraged to apply. Only short-listed candidates will be notified. Terms of reference and more information on the Fisheries Scientist position and other vacancies can be obtained from the MRC website **www.mrcmekong.org**

FELLOWSHIPS

Friedrich-Schiller-University

Max Planck Institute for Chemical Ecology

International Max Planck Research School

"*The Exploration of Ecological Interactions with Molecular and Chemical Techniques*"

20 PhD Fellowships in Molecular and Chemical Ecology

The International Max Planck Research School (IMPRS) on

"*The Exploration of Ecological Interactions with Molecular and Chemical Techniques*"

in Jena, Germany, is the first graduate school worldwide where modern chemical and molecular techniques are systematically linked to ecological research.
The research school offers 20 PhD fellowships in molecular biology, ecology, entomology, microbiology or (bio)-chemistry, beginning in July 2006.
Students holding a Master's degree (or equivalent) from any national and international university with a proven record of success in one of the relevant disciplines and being interested in examining traits of ecological interactions are eligible to conduct a doctoral project within the IMPRS. Courses of the IMPRS are held in English.
Application deadline is **January 15, 2006.**

For detailed information on the IMPRS, the application and admission procedures in Jena please visit our website **http://imprs-jena.ice.mpg.de/**

MAX-PLANCK-GESELLSCHAFT

BIOLOGY FACULTY POSITIONS
San Diego State University

Two tenure track appointments are offered at the assistant professor level in the Biology Department to begin Fall 2006. Ph.D. required and post-doctoral experience preferred. Successful applicants will be expected to establish externally funded research programs involving B.S., M.S. and Ph.D. students and to interact with a diverse student body. Consideration will include the candidate's perceived match to our programmatic strengths including research emphases, teaching innovation, and student mentoring. Candidates should be able to interact with 14 full-time ecology faculty members with research emphases in coastal marine ecology, ecosystem/global change, and conservation/restoration ecology.

1. **Behavioral Ecologist.** Research interests must be field-based and could include elucidating behavioral processes to predict ecological patterns, using empirical and comparative analyses to relate behavior to the environment, conducting behavioral studies with relevance to conservation, and examining links between evolutionary adaptations and behavioral ecology. Teaching responsibilities to include graduate seminar/course in behavioral ecology and undergraduate courses in animal behavior and sociobiology. Periodic participation in undergraduate biostatistics and/or general ecology also desirable.

2. **Ecosystems Ecologist.** Desirable research interests include ecosystem carbon flux, nutrient dynamics, earth system science, sustainability, and/or modeling of ecosystem dynamics and distributions. Teaching responsibilities to include upper division/graduate courses in one's area of specialty, general ecology, biostatisics, and/or a non-majors course in environmental science.

More information on both positions is available at **http://www.sci.sdsu.edu/fac-recruitment**. Review of applications for both positions begins **December 1, 2005** and will continue until the positions are filled. Applicants for either position should submit a curriculum vitae, separate statements of research and teaching interests, 3 representative publications and arrange for 3 letters of recommendation to be sent to the **Behavioral Ecologist Search Committee or Ecosystem Ecologist Search Committee, Department of Biology, San Diego State University, San Diego, CA 92182-4614**.

SDSU is a Title IX, Equal Opportunity Employer and does not discriminate against individuals on the basis of race, religion, national origin, sexual orientation, gender, marital status, age, disability or veteran status, including veterans of the Vietnam era.

Molecular Geneticist

The Department of Forensic Sciences invites applications for a faculty position in forensic molecular biology at the level of **ASSISTANT PROFESSOR (5 year renewable contract)** Responsibilities include teaching and advising master's students and participating and developing research projects in forensic molecular biology. Qualifications include a Ph.D. in biology, genetics, molecular genetics or a related field and experience in human DNA marker testing. In addition, teaching and forensic laboratory experience including serological screening of evidence is desirable. Information on the department can be found at **http://www.gwu.edu/~forensic/**. This position offers the opportunity to work with scientists at The George Washington University, The Smithsonian Institution, NIST and federal and state agencies that collaborate with the Department of Forensic Sciences. The successful candidate will benefit from a competitive startup package. The starting date for the position is August 2006. Salary is commensurate with experience.

Application review will begin **January 3, 2006** and continue until the position is filled. Applicants should send a current vita, a summary of research interests, and arrange for three letters of reference to be sent to: **Dr. Moses S. Schanfield, Professor and Chair, Department of Forensic Sciences, Samson Hall 102, 2036 H Street, NW, Washington, DC 20052**.

The George Washington University is an Equal Opportunity / Affirmative Action Employer.

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Professor / Assistant Professor (Tenure Track) in Pharmaceutical Chemistry / Chemical Genetics

The new professor will join the Institute of Pharmaceutical Sciences within the Department of Chemistry and Applied Biosciences. To complement existing strengths of the Institute, the candidate's research should be positioned in the field of computational pharmaceutical chemistry and/or include the structural investigation and biophysical characterization of protein-ligand-complexes and/or the identification of lead structures for drug discovery.

Candidates should have a record of outstanding scientific achievements and present a well-developed, novel and creative research program with a clear emphasis on biomedical / pharmaceutical questions. They should be clearly committed to interdisciplinary research in an interdisciplinary environment. Teaching in the field of Pharmaceutical Chemistry is an integral part of this chair. ETH has a commitment to excellence in teaching and supports modern teaching methods. The rank of the position (full or assistant professor) will depend on the applicant's qualifications and expertise. Courses at Master level may be taught in English.

Assistant professorships at ETH Zurich have been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

Applications with a curriculum vitae, a list of publications and an outline on future teaching and research plans should be sent **to the President of ETH Zurich, ETH Zentrum, CH-8092 Zurich, Switzerland, no later than January 15, 2006**. ETH Zurich specifically encourages female candidates to apply with a view towards increasing the proportion of female professors.

New Faculty Positions
Monmouth University - Department of Biology

Assistant or Associate Professor
Marine or Environmental Biology

A Ph.D. in marine biology, environmental biology or related area, a minimum of two years of postdoctoral experience (assistant professor) or 7 years faculty experience (associate professor), and research experience in marine, aquatic or estuarine biology, are required. Also required are a commitment to undergraduate education, evidence of past teaching effectiveness and ability to direct undergraduate research. Preference will be given to candidates with experience managing and conducting grant-funded research and to candidates best able to contribute to a new undergraduate program in Marine & Environmental Biology and Policy, a Center for Coastal Watershed Management, and the new Urban Coast Institute.

Lecturers - Two full time renewable positions

Required are a Ph.D. in a biological science and a commitment to undergraduate education. Preference will be given to candidates with teaching experience and documentation to support past teaching effectiveness at the college/university level. Candidates best able to teach clusters of courses chosen from: an introductory course for majors (focused on cellular and molecular biology, biochemistry and genetics), ecology, botany, vascular plants, biochemistry, marine biology and evolution, are preferred. Candidates may also be expected to teach a course for non-majors.

Submit a cover letter addressing the requirements and preferences specified in this ad, a curriculum vitae, documentation to support past teaching effectiveness, (a research plan for assistant/associate professor), and a minimum of two letters of recommendation to Dean Francis Lutz, School of Science Technology & Engineering, Monmouth University, West Long Branch, NJ 07764. Deadline for postmark on all application materials is December 15, 2005. Starting date for all positions is expected to be September 2006.

MONMOUTH UNIVERSITY

where leaders look forward℠

MONMOUTH UNIVERSITY IS AN EQUAL OPPORTUNITY, AFFIRMATIVE ACTION EMPLOYER.

UMDNJ - NEW JERSEY MEDICAL SCHOOL - UNIVERSITY HOSPITAL CANCER CENTER

FACULTY POSITIONS IN A NEW CANCER CENTER

With the ongoing construction of the 220,000 square foot New Jersey Medical School (NJMS) - University Hospital (UH) Cancer Center, NJMS is now looking to undergo major expansion through aggressive recruitment of outstanding scientists in cancer research, at both the junior and senior level. This state-of-the-art facility will combine three clinical floors, including outpatient services, with four research floors, including core facilities, such as digital imaging and microscopy, a 20,000 sq. ft. mouse barrier facility, a proteomics facility and a tissue culture core. Additional core facilities including bioinformatics, transgenics, applied genomics and flow cytometry, are conveniently situated in adjacent buildings.

Successful candidates must have an M.D. and/or Ph.D. with outstanding credentials and will be expected to maintain a vigorous, independent extramurally funded research program. Generous start-up funds, competitive salary, and ultra-modern laboratory space are available for both tenured and tenure track positions. Although recruitment is expected to continue over several years, we are particularly interested at this time in those scientists prepared to move upon the opening of the facility in August 2006.

Applications, including curriculum vitae, three letters of reference and a brief summary of accomplishments and future research directions, should be sent to: **Harvey L. Ozer, M.D., Professor and Director, NJMS-UH Cancer Center, UMDNJ-New Jersey Medical School, 185 South Orange Ave., Newark, N.J. 07101.** Senior investigators need not provide letters of reference at this time and their applications will be treated with confidentiality if requested. For more information about the NJMS-UH Cancer Center visit: **http://njmsuhcc.umdnj.edu/research.** UMDNJ is an AA/EOE, M/F/D/V Employer. The Cancer Center is an Affiliate of the UMDNJ Cancer Institute of New Jersey.

UMDNJ
NEW JERSEY MEDICAL SCHOOL
UNIVERSITY HOSPITAL
CANCER CENTER
UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY

Science
AAAS

Genetics Editor at *Science*

Join the dynamic team at *Science* as a full-time associate editor for the biological sciences in our Washington, DC, USA or Cambridge, UK office. We are looking for a life scientist with broad interests, a lively curiosity, and experience in cutting-edge research in several of the following fields: genetics, genomics, evolution, evo-devo, and ecology. Responsibilities include managing the review, selection, and editing of manuscripts, soliciting reviews and special issues, and fostering contacts and communication with the scientific community. Editors are expected to travel to scientific meetings. A Ph.D., postdoctoral experience, and multiple publications are required. Previous editorial experience is not necessary.

For consideration, send a resume and cover letter, along with salary requirements, to:

AAAS
Human Resources Department, Suite #101
1200 New York Avenue
Washington, DC 20005

Applications can also be sent by e-mail to **hrtemp@aaas.org** or Fax to **202-682-1630**.

Visit us at: **www.aaas.org**.

Nonsmoking work environment. EOE.

Georgia State University

NEUROSCIENCE AND COMPUTATIONAL BIOMEDICINE

BRAINS and BEHAVIOR: The Brains and Behavior Program (B&B) at Georgia State University (**http://brainsbehavior.gsu.edu/**) offers graduate fellowships for its interdisciplinary initiative in neuroscience and behavior. The B&B Program brings together seventy faculty members from eight participating departments, Biology, Chemistry, Computer Science, Computer Information Systems, Mathematics and Statistics, Philosophy, Physics and Astronomy, and Psychology, to conduct collaborative research and graduate training. Interdisciplinary research groups within B&B include Neurons and Networks, Molecules and Brains, and Adaptability and Social Behavior. Each B&B Fellow will matriculate in a member department and be jointly supervised by a neuroscientist and a member from their home department. The Brains and Behavior Program is affiliated with the Center for Behavioral Neuroscience (**http://www.cbn-atl.org/research/index.cfm**), a National Science Foundation Science and Technology Center.

MOLECULAR BASIS OF DISEASE: The Molecular Basis of Disease Area of Focus (MBD) at Georgia State University, Atlanta, GA, (**biology.gsu.edu/MBD**) is recruiting students for its newly established Ph.D. fellowship program. The MBD Area of Focus is an interdisciplinary program in computational biomedicine that includes over seventy faculty members in the Departments of Biology, Chemistry, Computer Science, Physics and Astronomy, Mathematics and Statistics, and Computer Information Systems. Interdisciplinary research foci within the MBD include Structural Biology, Computational Biology and Bioinformatics, Cancer and Infectious Diseases. Applications should be made directly to the Ph.D. programs of the participating departments. MBD fellows receive an annual stipend of $22,000 plus a full tuition waiver.

INFORMATION: For more information and to request application materials, please contact **Ms. Tara Alexander, biotea@langate.gsu.edu, phone: 404-463-9456.**

COMPUTATIONAL BIOLOGIST

PHYSICAL BIOSCIENCES DIVISION

Lawrence Berkeley National Laboratory (Berkeley Lab) is located in the San Francisco Bay Area on a 200-acre site in the hills above the University of California's Berkeley campus and is managed by the University. A leader in science and engineering research for more than 70 years, Berkeley Lab is the oldest of the U.S. Department of Energy's National Laboratories.

This exciting position is an engineering and research appointment responsible for the management and execution of a number of computational biology projects in a laboratory setting with the Physical Biosciences Division. Under the direction of Adam Arkin, PI, the incumbent will be the Computational Core technical co-lead on the VIMSS DOE: Genomics GTL project at Berkeley Lab. The incumbent will develop software packages for biological data and network analysis and help work with post docs, graduate students and technical staff. The position requires the ability to lead teams in bioinformatic software design and engineering, mathematical analysis and simulation of models, and formulation of research issues and plans. In addition, the incumbent will be expected to design and carry out research with the Directors of VIMSS. He/she will act as a liaison with other performers in the institute, other research programs, and JGI. It will be necessary to continue development of a comparative genomics suite of analyses and software, and prepare presentations and manuscripts for internal use and peer reviewed publications.

The Computational Biologist must have demonstrated high-level skills, knowledge and work experience in computational biology, especially in regard to comparative and functional genomics and genetic networks. Demonstrated ability to work collaboratively with programmers, engineers, post-docs, graduate students, and undergraduates from multiple backgrounds will also be important. He/she must have the ability to handle multiple projects and tasks in parallel, and meet programmatic milestones and project timelines. Demonstrated experience with high-level problem-solving in computational biology is required. Post-doctoral positions are also available.

For immediate consideration, apply online at: **http://jobs.lbl.gov**, select "Search", and enter 017775 in the keyword search field. Select "Search Jobs", and follow the on-line instructions to complete the application process. Enter "Science" as your source.

Berkeley Lab is an Affirmative Action/Equal Opportunity Employer committed to the development of a diverse workforce.

For more information about Berkeley Lab and its programs, visit **www.lbl.gov**.

FACULTY POSITIONS IN STRUCTURAL BIOLOGY

Department of Biochemistry and Molecular Biology
Kimmel Cancer Center
Thomas Jefferson University

The Department of Biochemistry and Molecular Biology and the Kimmel Cancer Center are seeking qualified candidates for junior and senior tenure-track faculty positions. The focus of the KCC, an NCI-designated cancer center, is to make basic science discoveries in cancer and translate them into novel modalities for prevention, diagnosis and treatment of cancer. Investigators are supported in part by core facilities (nucleic acids, protein chemistry/molecular interaction, proteomics, bioimaging, x-ray crystallography, microarray, transgenic/gene targeting, and pathology) within the Kimmel Cancer Center and by several NIH supported institutional training grants.

Qualified candidates should have a Ph.D. and/or M.D., and post-doctoral experience in x-ray crystallography and an interest in cancer as it relates to macromolecular structure and function. Faculty members are expected to establish an independent research program and interact with investigators within the University. Qualified candidates should send a curriculum vitae, a statement of current and future research plans, and request for three letters of recommendation to be sent to: **Dr. Jeffrey Benovic, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, 233 South 10th Street, Philadelphia, PA 19107.**

Thomas Jefferson University

Thomas Jefferson University is located in center city Philadelphia, adjacent to a variety of cultural, entertainment and historical attractions. Affirmative Action/Equal Opportunity Employer.

GRADUATE PROGRAM

University of California
San Diego

New Ph.D. program in PLANT SYSTEMS BIOLOGY at UCSD, SALK Institute and TSRI NSF IGERT GRADUATE TRAINING PROGRAM

This entirely new interdisciplinary training program will train graduate students with different backgrounds at the interface of biological systems modeling, computational genomics and plant sciences and will position Ph.D. students at the frontier of systems biology. The program will include focused mentoring of each student in two labs by two advisors from distinct disciplines (e.g. Systems Engineering/Bioinformatics and Plant Biology). 30 internationally leading laboratories in diverse disciplines are participating in this new program. For further information see: **http://biology.ucsd.edu/psbigert** .

Highly qualified candidates with diverse backgrounds and degrees in computer sciences, engineering, biology, physics, biophysics, mathematics, chemistry, or related subjects are invited to apply no later than **December 15, 2005**. On-line Application submission preferably to **http://www.biology.ucsd.edu/grad/index.html** or alternatively applicants can apply to the UCSD Bioengineering or Computer Sciences and Engineering Graduate Programs. Each application should indicate your interest in the Plant Systems Biology Program. For further information contact: Program Directors **Julian Schroeder (JISchroeder@ucsd.edu)** and **Steve Briggs (SBriggs@ucsd.edu)**.

SIU

TWO POSTDOCTORAL POSITIONS are available immediately to study neuroendocrine control of prolactin secretion and cellular/molecular regulatory mechanisms in hypothalamic neurons. Qualified applicants will have a Ph.D. in physiology or related field preferably with experience in neuroendocrinology and molecular biology. Review of applications will begin immediately and continue until the position is filled.

Send curriculum vitae and names of three references to:

Dr. Lydia Arbogast
Department of Physiology
Southern Illinois University
School of Medicine
Carbondale, IL 62901-6523

Women and minority applicants are encouraged to apply. SIU is an affirmative action/equal opportunity employer that strives to enhance its ability to develop a diverse faculty and staff and to increase its potential to serve a diverse student population. All applicants are welcomed and encouraged and will receive consideration.

McLaughlin Research Institute for Biomedical Sciences

POSTDOCTORAL POSITION

Postdoctoral positions are available immediately to study the regulation of developing cranial sensory neurons in the **mammalian** nervous system, molecular genetics of inner ear and kidney development at the McLaughlin Research Institute, a private institute that specializes in mouse genetics (**http://www.montana.edu/wwwmri**). The focuses of the research projects are to investigate the roles of transcription factors in the regulation of sensory neuron, auditory system or kidney development by generating and analyzing transgenic and knockout mice. Our recent publications are: *Dev. Biol*. **284:**323-336, 2005; *Development* **131:**5561-5572, 2004; *Proc. Natl. Acad. Sci. USA.* **101:**8090-8095, 2004; *Development* **130:**3085-3094, 2003; *Development* **130:**3989-4000, 2003.

McLaughlin research Institute has made significant contributions to mouse genetics for over 45 years, and benefits from an active Scientific Advisory Committee. The Institute provides a unique environment in which to use mice to study important problems in neurobiology and mammalian organogenesis. To apply, please send CV and three letters of reference to: **Dr. Pin-Xian Xu, McLaughlin Research Institute, 1520 23rd Street South, Great Falls, MT 59405, USA** or Email: **pxu@po.mri.montana.edu**.

An Equal Opportunity/Affirmative Action Employer.

Advanced Light Source Division Director

Lawrence Berkeley National Laboratory is a multi-purpose research laboratory, operated by the University of California for the Department of Energy. The Advanced Light Source (ALS) at LBNL is the leading national user facility for VUV and soft X-ray science, with additional outstanding capabilities from the infrared to the hard X-ray regions of the electromagnetic spectrum. The 1.9 GeV storage ring feeds 35 simultaneously operating beamlines, and is currently being upgraded. The state-of-the-art facilities serve over 2000 scientists annually in the fields of condensed matter, AMO physics, nanoscience and technology, surface chemistry, microscopy, spectroscopy, environmental science, macromolecular crystallography, geophysics and related fields. (**http://www-als.lbl.gov/**)

The Division Director is responsible for developing and articulating the vision of ALS, while maintaining its scientific leadership, high level of scientific productivity, and excellence of the user program. The individual will manage 180 career professional employees and approximately 2,000 users (guests) annually. Total operating budget is $40M/Year. The ALS Division Director must be committed to its safe and reliable operation. The holder of this position participates in the highest levels of management at LBNL.

The Laboratory is seeking a highly respected and energetic scientist, with experience and demonstrated superior achievement in Materials Sciences, Physics, Chemistry, Biology, or related science, and capable of providing strong scientific and intellectual leadership for the ALS. Candidates should have demonstrated successful leadership overseeing a multidisciplinary group of scientists and their accompanying support staff.

Applications are accepted online at **http://jobs.lbl.gov**. Select "Search" and enter 018488 in the keyword search field. Select "Search Jobs" and follow the instructions to complete the application process. Please submit (all as one document) a curriculum vitae, a list of publications, a statement of research accomplishments and interest, management experience, and the names of at least five references. Cover letters should be addressed to Dr. Paul Alivisatos, Chair, ALS Director Committee.

Lawrence Berkeley National Laboratory is an Equal Opportunity/Affirmative Action Employer, committed to a safe and diverse workforce. Applications will be considered until the position is filled. For more information about Berkeley Lab and its programs, visit **www.lbl.gov**.

BERKELEY LAB

CNRS CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

In 2006, CNRS is recruiting 410 tenured researchers in all scientific fields*.

This recruitment campaign is open **to junior and senior researchers from all over the world**. One of the major objectives of this campaign is to encourage international scientists to apply to CNRS.

CNRS researchers work in an enriching scientific environment:

- numerous large-scale facilities
- highly-skilled technical support
- multiple networks throughout Europe and across disciplines
- access to university research and teaching
- lab-to-lab and international mobility

At CNRS, a long-term vision of excellence in basic research provides a solid foundation for the latest technological research. Successful candidates from the CNRS competitive entry process benefit from the dynamics, stability and stimulation of belonging to a major research organization.

***Mathematics; Physics; Nuclear and High-Energy Physics; Chemistry; Engineering Sciences; Communication and Information Technology and Sciences; Earth Sciences and Astronomy; Environmental Sciences: Life Sciences; Humanities and Social Sciences.**

Application deadline: January 2006. Applications available online in December.

www.cnrs.fr

Research Corporation

PROGRAM OFFICER ROTATOR

Research Corporation is pleased to announce the establishment of a Rotator position for a Program Officer. The intent of this position is to provide mid-career scientists with the opportunity to learn the dynamics of the scientific foundation world and to fully participate in the rewarding processes of funding science. We seek individuals who want to "make a difference" in the scientific world in a manner that complements, yet also extends beyond, their own research and teaching.

At Research Corporation the Rotator will work as a fully functioning Program Officer and participate completely in all decision-making processes. In addition, the opportunity to work on a special project will be made available. Such a project would be framed from an integration of both the needs of the Foundation and the interests of the individual.

Each Rotator position will be for one year. We are expecting applicants to be individuals who seek a different, unique and rewarding opportunity for intellectual pursuits during their sabbatical year. Research Corporation will pay up to 50 percent of the annual salary of the Rotator and will also provide some relocation allowances. We seek in particular individuals with expertise in research areas that are at the interface of the physical sciences and the life sciences (i.e., biophysics, biochemistry, molecular biology, cell biology, etc.) and a demonstrated interest in science education. No previous academic administrative experience is necessary.

Knowing that the planning of a sabbatical leave for any individual takes time, we are open to conversations with individuals who anticipate a sabbatical leave from their institution anytime within four years from the date of initial inquiry.

The foundation's offices are located in Tucson, a vibrant Southwestern community of more than one-half million residents, surrounded by mountains and the Sonoran Desert, served by an international airport, with a major university, significant programs in the creative and performing arts, and nearly unlimited opportunities for outdoor activities.

More information about Research Corporation programs can be found on its website (**http://www.rescorp.org**). Application should be sent, preferably via e-mail, and should include vitae, the names and addresses of no more than five individuals who could serve as references, and a one-page statement that addresses why the applicant would find a Rotator position advantageous to her/his career goals. Applications will be reviewed as received. For more detailed information about this position as well as work expectations and opportunities, contact **Dr. Ray Kellman, Vice President at Research Corporation (ray@rescorp.org)**.

Research Corporation is an Equal Opportunity Employer.

ASSISTANT PROFESSOR ENGINEERED BIOLOGICAL SYSTEMS

The Biological Engineering Division invites applications for a tenure-track faculty position at the assistant professor level in Engineered Biological Systems, to begin July 2006 or thereafter. Applicants should hold a Ph.D. in a science or engineering discipline related to biological engineering. In special cases, a more senior faculty appointment might be possible. The candidate is expected to integrate strong expertise in molecular/cellular bioscience with an engineering design perspective; example areas of application might include stem cell technologies, vaccine development, biomolecular materials, and tissue engineering or synthetic biology broadly. We especially encourage minorities and women to apply, because of MIT's strong commitment to diversity in engineering education, research and practice.

Interested candidates should send application materials to be-fac-search@mit.edu. Each application should include: a curriculum vitae; the names and addresses of three or more references; a strategic statement of research interests; and a statement of teaching interests, specifically in the context of the Biological Engineering graduate and undergraduate educational programs at MIT (http://web.mit.edu/be/education/ and http://web.mit.edu/be/education/ugrad.htm).

We request that each candidate arrange for the reference letters to be sent directly to be-fac-search@mit.edu, with a copy mailed or faxed to the following address: Professor Paul Matsudaira, Chair, Faculty Search Committee, Biological Engineering Division, Massachusetts Institute of Technology, Bldg NE47, Room 223, 77 Massachusetts Avenue, Cambridge, MA 02139-4307. Fax#: 617-258-7226. Responses by 1 February 2006 will be given priority.

Massachusetts Institute of Technology

web.mit.edu/hr

STANFORD UNIVERSITY SCHOOL OF MEDICINE DEPARTMENT OF GENETICS

invites applications for a tenure-track position at the

ASSISTANT/ASSOCIATE/FULL PROFESSOR level

We seek highly interactive candidates with an outstanding record of achievement in the areas of modern genetics and genomics research. We are particularly interested in candidates with laboratory-based programs focused on current problems in human genetics. The Department of Genetics at the Stanford University School of Medicine offers a highly collegial and interdisciplinary environment that spans clinical medicine, model organism genetics, and genome-scale resources. See **http://genetics.stanford.edu** for more information.

Candidates should have a Ph.D. and/or M.D. degree, and substantial postgraduate experience. In addition to establishing and maintaining an independent research program, the successful candidate will actively participate in teaching and training medical and graduate students in the biomedical sciences.

Candidates are encouraged to apply by **February 1, 2006** with electronic documents that include a curriculum vitae, teaching experience, a statement of research interests and future research plans, and contact information for three or more individuals willing to serve as references, to: **faculty-search-z474@genome.stanford.edu**.

Greg Barsh, M.D., Ph.D.
Professor of Genetics and Pediatrics
Department of Genetics
Stanford University School of Medicine
Beckman Center for Molecular and Genetic Medicine
Stanford, CA 94305-5323
email: faculty-search-z474@genome.stanford.edu

Stanford University is an Equal Opportunity, Affirmative Action Employer.

VICE CHANCELLOR FOR RESEARCH

UCI
University of California, Irvine

The University of California, Irvine, invites applications and nominations for the position of Vice Chancellor for Research who will provide leadership and vision for future research growth and development at the University of California, Irvine (UCI).

We seek an individual with an abiding commitment to the excellence of research at a top tier research university. The principal responsibility is to foster and support the continued development of the research infrastructure, including the development of core funding and organized research centers of excellence. The ability to cultivate positive and productive collaborative relationships with diverse faculty and administrators is essential. A key role involves representing the campus in matters related to research to the Office of the President, other UC campuses, other research universities, federal, state and private agencies, the local community, and the media. The Vice Chancellor for Research is also responsible for oversight of campus activities in contract and grant administration, technology transfer, research administration (human/animal), integrity in research, conflict of interest, and research resource development. The Vice Chancellor for Research has fiscal and administrative responsibility for the Office of Research, which has a staff of approximately 325 employees, with over 100 in the central Office of Research administration and 225 in the research units.

Candidates must be qualified for appointment at the rank of full professor in an academic discipline. Salary is competitive and commensurate with background and qualifications.

Founded in 1965, UCI is one of ten campuses of the prestigious University of California system. The campus is located on a 1500-acre site, three miles from the ocean in Orange County, approximately 40 miles south of Los Angeles.

The position will remain open until filled. The search committee will begin consideration of applications and nominations on **January 1, 2006**. A Curriculum Vitae should be sent electronically to: **vcsearch@uci.edu**. Alternative address for submission is: **Vice Chancellor for Research Search Committee, C/O Cynthia Hoffman, UCI - 535B Administration Building, Irvine, CA 92697-1000.**

UCI is an Equal Opportunity Employer committed to excellence through diversity.

Colby

Environmental Studies/ GIS and Policy

The Environmental Studies Program at Colby College invites applications for a one-year position as Faculty Fellow in Environmental Studies beginning **September 1, 2006.** The successful candidate will teach courses in domestic environmental policy and introductory Geographic Information Systems (GIS) with a laboratory, as well as one in an area of her/his environmental studies specialty. Candidates should have a Ph.D. in environmental studies or a related field and a strong commitment to undergraduate education. Familiarity with liberal arts colleges and teaching experience are desirable. Colby offers a highly competitive salary. For more information about Colby's Environmental Studies Program visit: **www.colby.edu/environ/**. Review of applications will begin on **December 1, 2005** and continue until the position is filled. Please submit a letter of application, a statement of teaching interests and experience, a curriculum vitae, three reference letters, and representative teaching evaluation summaries, if available, to: **Professor Russell Cole, Chair, Environmental Studies Search Committee, 5728 Mayflower Hill, Waterville, ME 04901.**

Colby is an Equal Opportunity/Affirmative Action employer, committed to excellence through diversity, and strongly encourages applications and nominations of persons of color, women, and members of other under-represented groups. For more information about the College, please visit the Colby web site:

www.colby.edu

Geneticist/Molecular Biologist/Immunologist/Microbiologist/Virologist
St. Cloud State University
St. Cloud, MN
Biological Sciences

The Department of Biological Sciences anticipates filling three tenure track faculty positions at the Assistant or Associate Professor level for the academic year 2006-2007. The successful candidate will possess an earned Ph.D. in the Biological Sciences or related field with post-doctoral experience. Preference given to candidates with successful post-secondary teaching experience and a strong commitment to undergraduate instruction. Incumbents will have shared responsibilities in teaching a subset of the following courses depending on qualifications and interests: microbiology (for health professionals and for majors), virology, immunology, pharmacology, pathophysiology (for majors), genetics, parasitology, hematology, neurobiology, plant tissue culture, advanced DNA techniques, advanced protein techniques and/or introductory major's courses. A research program that complements existing department faculty interests (see department website) and has demonstrated potential for extramural funding is preferred. This research program will be expected to contribute to professional development and involve undergraduate and graduate students. Advising and committee participation are expected. Faculty will be required to document the following for promotion and tenure: ability to teach effectively, perform scholarly achievement or research, continued preparation and study, contribution to student growth and development and service to the university and community. All candidates will be expected to demonstrate the ability to teach and work with persons from culturally diverse backgrounds.

Send letter of application including statement of research plans, teaching philosophy, courses the applicant is most suited or interested in teaching from this ad, curriculum vitae, transcripts (copies acceptable for initial screening), and the name, phone number, postal and e-mail address of three references. We will contact references to comment specifically upon your teaching ability, experience and professional preparation. Submit materials to: **Chair, Department of Biological Sciences, St. Cloud State University, 720 4th Avenue South, St. Cloud, MN 56301-4498.** You may contact us by phone, **(320) 308-5433, FAX, (320) 308-4166**, or e-Mail, **Biology@StCloudState.edu** or **www.StCloudState.edu/~biol**. All materials must be postmarked by **23 December 2005** to be considered. E-mail or FAXed applications received after 23 December 2005 may not receive consideration.

SCSU is committed to excellence and actively supports cultural diversity. To promote this endeavor, we invite individuals who contribute to such diversity to apply, including minorities, women, GLBT, persons with disabilities and veterans. SCSU is a member of the Minnesota State Colleges and University System (MnSCU).

Research Corporation

PROGRAM OFFICER

Research Corporation is seeking a talented mid-career scientist with expertise in the life sciences or materials sciences to assist the foundation in its support of science, and scientists and their work. Responsibilities include processing and evaluating research proposals, evaluating one-of-a-kind opportunities in science and science education, visiting colleges and universities on behalf of the foundation, representing the foundation at professional meetings, and assessing trends and opportunities in the physical and life sciences. Applicants must have a Ph.D., preferably in chemistry, biochemistry, biophysics, or physics; excellent communication skills; the ability to work well with others, including a wide range of scientists and university administrators; and an interest in working across the disciplines in the physical and life sciences. Applicants must have five or more years of experience as a faculty member or an industrial/government scientist and a demonstrated record of accomplishment within this setting. In particular, a successful record of grantsmanship and/or independent publication is expected. Salary is commensurate with experience and qualifications and includes an outstanding package of benefits.

The foundation's offices are located in Tucson, AZ, a vibrant Southwestern community of more than one-half million residents, surrounded by mountains and the Sonoran Desert, served by an international airport, with a major university, significant programs in the creative and performing arts, and nearly unlimited opportunities for outdoor activities.

More information about Research Corporation programs can be found at the foundation website (**http://www.rescorp.org**). Application should include vitae, the names and addresses of no more than five individuals who could serve as references, and a one-page statement that addresses why the applicant would undertake a career in science advancement. Applications will be reviewed as received. Applications, preferably via e-mail, should be sent to the attention of: **Dr. Ray Kellman, Vice President at Research Corporation (ray@rescorp.org)**.

Research Corporation is an Equal Opportunity Employer.

FELLOWSHIPS

Smithsonian Tropical Research Institute

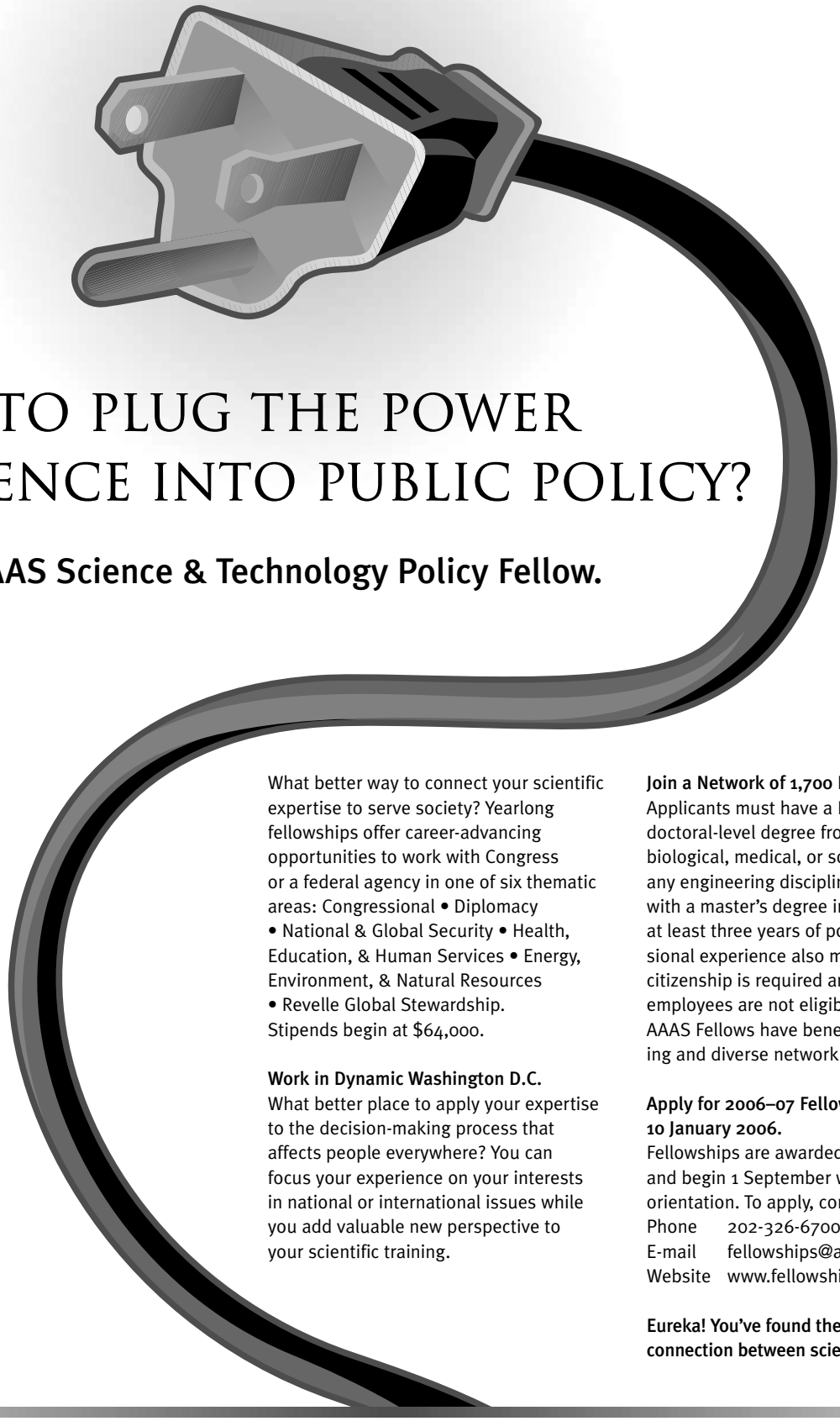
Fellowships in Tropical Biology

The Smithsonian Tropical Research Institute (STRI), a division of the Smithsonian Institution located in the Republic of Panama, maintains a series of research facilities in different marine and terrestrial locations on the Isthmus of Panama. STRI offers fellowships for undergraduate, predoctoral and postdoctoral research in the areas represented by its scientific staff. Disciplines include ecology, anthropology, paleontology, evolutionary biology, molecular phylogenetics, biogeography, animal behavior, soil sciences, physiology of tropical plants or animals, neurobiology and paleoecology.

- **Earl S. Tupper 3-year postdoctoral fellowship** (deadline: Jan15) Applications should include detailed research proposal with budget, curriculum vitae, 2 letters of reference, names and telephone numbers of 3 additional references. Applicants should consult with STRI scientists who will serve as advisors before submitting final application. Annual stipend of $35,000 plus research allowance. Send inquiries and application to **STRI/Office of Academic Programs, Unit 0948, APO AA 34002-0948, from US or Apartado 0843-03092, Balboa, Panama from Latin America, fellows@si.edu**.
- **Predoctoral, postdoctoral, senior postdoctoral** (up to 1 year) and 10-week fellowships. Available through the Smithsonian's Office of Fellowships, Washington, DC. (deadline: Jan15). For information: **OFG, 750 9th Street NW, Suite 9300, Washington DC 20560-0902, siofg@ofg.si.edu, www.si.edu/research+study**.
- **Marine postdoctoral fellowships** (deadline: Jan15) For comparative research of the Atlantic and Pacific coasts of the Republic of Panama. Preference will be given to projects using STRI's research station in Bocas del Toro. Applications should include detailed research proposal with budget, curriculum vitae, 2 letters of reference, names and telephone numbers of 3 additional references. This opportunity may be extended to 2 years. Applicants should consult with STRI scientists who will serve as advisors before submitting final application. Send inquiries and application to **STRI/Office of Academic Programs, Unit 0948, APO AA 34002-0948, from US or Apartado 0843-03092, Balboa, Panama from Latin America, fellows@si.edu**.

Awards are based upon merit, without regard to race, color, religion, sex, national origin, age or condition of handicap of the applicant.

For more information, visit: **http://stri.org**



WANT TO PLUG THE POWER OF SCIENCE INTO PUBLIC POLICY?

Become a AAAS Science & Technology Policy Fellow.

What better way to connect your scientific expertise to serve society? Yearlong fellowships offer career-advancing opportunities to work with Congress or a federal agency in one of six thematic areas: Congressional • Diplomacy • National & Global Security • Health, Education, & Human Services • Energy, Environment, & Natural Resources • Revelle Global Stewardship. Stipends begin at $64,000.

Work in Dynamic Washington D.C.
What better place to apply your expertise to the decision-making process that affects people everywhere? You can focus your experience on your interests in national or international issues while you add valuable new perspective to your scientific training.

Join a Network of 1,700 Fellows.
Applicants must have a PhD or equivalent doctoral-level degree from any physical, biological, medical, or social science, or any engineering discipline. Individuals with a master's degree in engineering and at least three years of post-degree professional experience also may apply. U.S. citizenship is required and federal employees are not eligible. Since 1973, AAAS Fellows have benefited from a growing and diverse network of colleagues.

Apply for 2006–07 Fellowships by 10 January 2006.
Fellowships are awarded in the spring and begin 1 September with a two-week orientation. To apply, contact AAAS:

Phone	202-326-6700
E-mail	fellowships@aaas.org
Website	www.fellowships.aaas.org

Eureka! You've found the perfect connection between science and policy.

Enhancing Public Policy, Advancing Science Careers

www.fellowships.aaas.org

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

FACULTY POSITIONS DEPT OF BIOLOGY

The Department of Biology at Yeshiva College, the men's undergraduate liberal arts college of Yeshiva University, invites applications for two tenure-track faculty positions at the level of Assistant or Associate Professor to begin Fall 2006. Successful candidates are expected to develop strong, externally funded, experimental research programs involving undergraduates and to contribute to the teaching mission of the Department. Areas of interest include, but are not limited to, developmental biology, stem cell research and macromolecular structure and function.

Applicants should submit curriculum vitae, separate two-page statements of research and teaching interests, up to three scientific papers, and arrange for three letters of recommendation to be sent to: **Biology Search Committee, Office of the Dean, Yeshiva College of Yeshiva University, 500 West 185th Street, New York, NY 10033-3312.** Review of complete applications will begin on **Dec. 1, 2005.** EOE

Yeshiva University

UCLM
UNIVERSIDAD DE CASTILLA-LA MANCHA
Centenario Don Quijote

RESEARCH POSITIONS CALL FOR APPLICATION

The University of Castilla - La Mancha (UCLM) invites applications from researches in different fields. The University offers 10 positions to researches with a solid background and recognized experience. The applicants must have earned a PhD degree before the year 2000, and must have held research positions outside of Spain for al least two years, be it as a doctoral or postdoctoral fellow.

The research areas in which UCLM is interested include, but are not limited to, **renewable energy**, **biotechnology** (specially biotechnology related to food science), **materials science**, **nanotechnology**, **environmental technology**, **communication and information technology**, and **automatic control and robotics**.

UCLM will offer the selected candidates a contract as a Visiting Professor for one year, followed by the possibility to either extend said contract or to permanently join UCLM in a tenured faculty position. The salary as Visiting Professor will be determined according to the candidate's merits and will be in the range of those corresponding to associate or full professors in Spanish universities.

Applications should be submitted online using the UCLM web page: **www.uclm.es** before December 31st, 2005.

The UNIVERSITY of VERMONT

Engaging minds that change the world

Chair of the Department of Surgery

The University of Vermont College of Medicine, in alliance with Fletcher Allen Health Care, is seeking both applications and nominations for outstanding candidates for the Stanley S. Fieber endowed Professorship and Chair of the Department of Surgery. The Chair will be responsible for clinical program development, medical student education, house staff training, and development of research programs.

The Chair also will serve as Clinical Leader of Surgery at Fletcher Allen Health Care, a health care system comprised of a 500-bed, acute care hospital, a new ambulatory care center, approximately 490 specialty and primary care physicians practicing at more than 40 sites throughout the region, and a total attending staff of approximately 685 physicians. In addition, the Chair will serve as department liaison with teaching hospital partner, Maine Medical Center, regarding faculty appointments and academic issues.

The University of Vermont College of Medicine, in partnership with Fletcher Allen Health Care, comprises the only quaternary academic medical center in the State and offers state of the art clinical, research and educational facilities. The Level 1 Trauma Center is verified by the American College of Surgeons in both adult and pediatric trauma care. The vital, growing faculty of the Department of Surgery includes 78 members within 13 divisions (including Emergency Medicine). The Department offers residency programs in general surgery and various subspecialties. It has active and established, extramurally funded, basic and clinical research programs in various disciplines.

The applicant should be Board Certified in Surgery with substantial experience in the administrative, teaching, clinical, and research activities of an academic department of surgery.

The University of Vermont and Fletcher Allen Health Care are located in Burlington, Vermont, a vibrant community located on the shores of Lake Champlain, between the Adirondack and Green Mountains. It offers four season recreational opportunities, has been cited as one of the most livable cities in the U.S., and a superb place to raise a family.

The University of Vermont is an Equal Opportunity/Affirmative Action Employer. Women and those from diverse racial, ethnic and cultural backgrounds are encouraged to apply. Applications will be accepted until the position is filled. A curriculum vitae and letter of interest should be sent electronically (if possible) to Linda Thatcher at the address below.

Dr. Edwin Bovill, Chair, Surgery Search Committee
c/o Linda Thatcher (linda.thatcher@uvm.edu)
E-126 Given Building, University of Vermont College of Medicine
Burlington, VT 05405-0068, Or apply online at www.uvmjobs.com

The University of Vermont is an Equal Opportunity/Affirmative Action Employer. Applications from women and people from diverse racial, ethnic, and cultural backgrounds are encouraged.

FELLOWSHIPS

AAAS 2006 Mass Media Science & Engineering Fellows Program

Are you interested in science writing?

Do you want to help people understand the impact and importance of discovery?

Work to increase public understanding of science and technology.

Fellows in the 10-week 2006 summer program will work as reporters, researchers, and production assistants in mass media organizations nationwide.

2005 host sites included:

Chicago Tribune, The Los Angeles Times, National Public Radio, Scientific American

For eligibility requirements, please visit ***http://ehrweb.aaas.org/massmedia.htm*** for more details and an application brochure, or call 202-326-6441 for more information.

DEADLINE JANUARY 15, 2006

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

POSITIONS OPEN

FACULTY POSITION
Molecular and Cellular Biology of Cancer

The Department of Pathology, Dalhousie University Faculty of Medicine, invites applications for a tenure-track appointment (**ASSISTANT/ASSOCIATE PROFESSOR**; rank commensurate with experience) from investigators whose research expertise is within the broad field of molecular and cell biology of cancer. Recent development within the Department includes the appointment of The Cameron Chair in Cancer Research, complementing the continued commitment to basic cancer biology research. Position qualifications include a Ph.D. and/or an M.D. (or equivalent degree) and postdoctoral experience. The successful applicant will have, or be expected to develop, an outstanding independent research program and will become part of a strong facultywide developing program focused on cancer research and training. This program includes basic and clinical scientists with interests spanning molecular, translational, clinical, and population-based research. Located in the beautiful Maritime environment and vibrant Halifax, Nova Scotia, we are home to some of Canada's top scientists, clinicians, and students and a centre of excellence that contributes much to the world's medical knowledge base.

The deadline for applications is January 31, 2006. Interested applicants please send curriculum vitae, a narrative of research and educational interests, and the names and contact information of three references to: **Christine Anjowski, Administrator, Department of Pathology, Dalhousie University, 5850 College Street, Halifax, Nova Scotia B3H 1X5. E-mail: c.anjowski@dal.ca.**

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. Dalhousie University is an Employment/Affirmative Action Employer. The University encourages applications from qualified Aboriginal people, persons with a disability, racially visible persons, and women.

ASSISTANT PROFESSOR, DEVELOPMENTAL BIOLOGY. The Biology Department of Coe College, a select midwestern liberal arts college with approximately 1,300 students, seeks a broadly trained Developmental Biologist. Teaching responsibilities will include developmental biology with laboratory and introductory biology courses with laboratory as needed by the Department. The successful candidate may develop an upper-division course in her/his area of expertise. Ph.D. required. Demonstrated interest and potential in undergraduate teaching and desire to supervise undergraduate research are important. Submit curriculum vitae, graduate transcripts, three letters of reference, and a statement of teaching and research interests to: **Dr. Marc Roy, Dean of the Faculty, Biology Search, Coe College, 1220 1st Avenue N.E., Cedar Rapids, Iowa, 52402.** To ensure full consideration, materials must be received by January 25, 2006. *Coe College is an Equal Opportunity/Affirmative Action Employer. Coe College is committed to increasing the diversity of the campus community and especially encourages applications that will fulfill that mission.*

MARINE ICHTHYOLOGIST

The University of New England (UNE) invites applications for a tenure-track **ASSISTANT** to **ASSOCIATE** level professorship in the Department of Biological Sciences to develop a funded research program, conduct research in UNE's Marine Science Center (MSC), and teach introductory biology and advanced courses in specialty areas.

Candidates with research interests that incorporate use of the MSC facility, study local fauna or flora, and complement existing departmental needs will be given preference.

Review of applications will begin in December 2005 and will continue until the position is filled. For qualifications, application, and contact information, see **website: www.une.edu/hr/vacancylist.asp.**

UNE is an Equal Opportunity/Affirmative Action Employer and strongly encourages candidates of diverse backgrounds.

POSITIONS OPEN

FACULTY POSITIONS
Department of Pharmacology and Toxicology
College of Pharmacy
University of Arizona Health Sciences Center

The Department of Pharmacology and Toxicology, College of Pharmacy, invites applications for two **ASSISTANT PROFESSOR** (job 33886) and one **ASSISTANT/ASSOCIATE/FULL PROFESSOR** (medicinal chemistry; job 33910) tenure-track faculty positions for the 2006–2007 academic year. For job 33886, individuals with interests in the areas of molecular pharmacology or molecular toxicology will be considered, but the following related areas are of particular interest during this phase of our expansion: apoptosis and stress response signal transduction pathways, chromatin structure and function, proteomics, and toxicogenomics (genetics, microarray technology, single nucleotide polymorphism analysis, and bioinformatics). For job 33910, we are seeking a synthetic medicinal chemist who has research interests in the design and synthesis of molecularly targeted therapeutics. An emphasis on cancer therapeutics is preferred, but other areas will also be considered. Our medicinal chemistry and pharmacology/toxicology faculty are building a strong program in drug discovery/development with translational goals being a major objective aligned with the Arizona Cancer Center and BIO5 Institute. Successful candidates will be expected to develop and/or maintain an independent, extramurally funded research program and to actively participate in undergraduate, graduate (Ph.D.), and professional (Pharm.D.) teaching. The Department will be undergoing a period of dynamic growth in parallel with facilities expansion. Excellent opportunities exist for participation in the Center for Toxicology, the Arizona Cancer Center, the BIO5, and other centers of excellence at the University of Arizona and the Arizona Health Sciences Center. Applications will be reviewed beginning December 1, 2005, and review will continue until the positions are filled.

For further information and to apply, please go to **website: http://www.hr.arizona.edu,** UofA jobs, Search Postings, job 33886 or job 33910. We offer excellent benefits and competitive salaries. *The University of Arizona is an Equal Employment Opportunity/Affirmative Action Employer. Minorities/Women/Persons with Disabilities/Veterans.*

TENURE-TRACK POSITION
Developmental Biology
Bowdoin College

The Biology Department at Bowdoin College invites applications for a tenure-track position in Developmental Biology at the **ASSISTANT PROFESSOR** level beginning fall 2006. We are seeking candidates who will demonstrate excellence in both teaching and research. Postdoctoral experience preferred. Typical teaching responsibilities each year include one laboratory course in developmental biology (with a laboratory instructor), one course at the nonmajors or introductory biology level, and one advanced course in one's area of research. The successful applicant is expected to pursue an active research program that involves undergraduates.

Review of applications will begin December 18, 2005, and will continue until the position is filled. Please send curriculum vitae with a description of your research interests and teaching philosophy and arrange to have three letters of reference sent to: **Search Committee Chair, Biology Department, 6500 College Station, Bowdoin College, Brunswick, ME 04011-8465.** For further information about the College, the Department, and the program, please see our **website: www.bowdoin.edu/biology/.**

Bowdoin College is committed to equality through Affirmative Action and is an Equal Opportunity Employer. We encourage inquiries from candidates who will enrich and contribute to the cultural and ethnic diversity of our College. Bowdoin College does not discriminate on the basis of age, race, creed, color, religion, marital status, gender, sexual orientation, veteran status, national origin, or disability status in employment, or in our education programs.

POSITIONS OPEN

ASSISTANT PROFESSOR
CELLULAR AND MOLECULAR BIOLOGY
TWO TENURE-TRACK POSITIONS

The Department of Biological, Geological and Environmental Sciences at Cleveland State University (CSU) invites applications for two tenure-track positions at the Assistant Professor level. These positions will complement a recent, focused expansion of faculty with robust research programs in cellular and molecular biology. Candidates with interests and expertise in any area of molecular and cellular biology, particularly those performing research in the area of molecular medicine, are encouraged to apply. For one position, the ability to teach microbiology is a plus. Applicants must hold a Ph.D., have postdoctoral or equivalent experience, and demonstrate the potential for excellence in research and teaching. The successful candidate will be expected to establish an independent, externally funded research program and participate in undergraduate and graduate teaching. The start date for both positions is August 21, 2006.

Before January 2, 2006, candidates must submit curriculum vitae, a description of research plans and teaching interests, and up to three reprints and must arrange for three letters of recommendation to be sent to: **Dr. Joseph D. Fontes, Chair, Faculty Search Committee, Department of Biological, Geological and Environmental Sciences, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115-2214.** Applications will also be accepted as PDF attachments to **e-mail: j.fontes@csuohio.edu;** letters of recommendation must be sent by post.

The Department offers B.S. and M.S. degrees and the Ph.D. degree through a joint program with the Lerner Research Institute of the Cleveland Clinic Foundation. For additional information, consult the Department's **website: http://web.bges.csuohio.edu** and the Lerner Research Institute **website: http://www.lerner.ccf.org** or contact: **Dr. Fontes, e-mail: j.fontes@csuohio.edu; telephone: 216-523-7199.** *CSU is an Affirmative Action/Equal Opportunity Employer Institution committed to nondiscrimination in employment and education. Minorities/Females/Persons with Disabilities/Veterans encouraged.*

ASSISTANT/ASSOCIATE PROFESSOR
Department of Medicinal & Biological Chemistry
The University of Toledo

The University of Toledo seeks an Assistant/Associate Professor with a Ph.D. in medicinal chemistry, organic chemistry, or a related field for an appointment in the Department of Medicinal and Biological Chemistry in the College of Pharmacy. The candidate will be expected to teach undergraduate- and graduate-level courses in medicinal and biological chemistry.

The candidate will be required to develop and maintain an independent, externally funded research program. The research area preferred will incorporate diversity-oriented synthesis and target-directed chemoinformatics approaches in drug design, emphasizing pharmacogenomic/chemical genetics strategies when appropriate. A competitive salary and startup package will be provided. This is a 12-month position that can start July 1, 2006.

Please send curriculum vitae with a description of future research plans and arrange for three letters of reference to be sent to:

Chair of Search Committee
Department of Medicinal & Biological Chemistry
The University of Toledo
College of Pharmacy
Room BO 2830
2801 W. Bancroft Street
Toledo, OH 43606

Review of completed applications begins January 15, 2006, and will continue until the position has been filled. Further information may be obtained at **website: http://www.mbc.pharm.utoledo.edu.**

The University of Toledo is an Equal Access, Equal Opportunity, Affirmative Action Employer and Educator.

The Santa Fe Institute
2006 Complex Systems Summer Schools

SANTA FE: June 4-30, 2006, in Santa Fe, New Mexico, USA.
Administered by the Santa Fe Institute (SFI).
Director: Dr. Dan Rockmore, Dartmouth College and SFI.

BEIJING: July 9 to August 4, 2006, in Beijing, China. Sponsored by SFI in cooperation with The Institute of Theoretical Physics, Chinese Academy of Sciences (CAS). Co-directors: Dr. David P. Feldman, College of the Atlantic and SFI, and Dr. Chen Xiao-song, Institute for Theoretical Physics, CAS.

The Complex Systems Summer School offers an intensive four-week introduction to complex behavior in mathematical, physical, living, and social systems for graduate students and postdoctoral fellows. The schools are aimed at participants who want to obtain the background and hands-on experience that will help prepare them to do interdisciplinary research in areas related to complex systems.

Each school consists of an intensive series of lectures, laboratories, and discussion sessions focusing on foundational ideas, tools, and current topics in complex systems research. These include nonlinear dynamics and pattern formation, scaling theory, information theory and computation theory, adaptation and evolution, network structure and dynamics, adaptive computation techniques, computer modeling tools, and specific applications of these core topics to various disciplines. In addition, participants will formulate and carry out team projects related to topics covered in the school.

Participants are expected to attend one school for the full four weeks. No tuition is charged, and some support for housing and travel expenses is available. Enrollment is limited.

TO APPLY: Applications are solicited from graduate students and postdoctoral fellows in any discipline. Some background in science and mathematics is required, as well as English language proficiency. Applicants may apply to either the Santa Fe or Beijing school, regardless of home country; however, placements may be influenced by restrictions in U.S. foreign visitor policies.

Please see SFI's website at http://www.santafe.edu/csss06.html for complete eligibility requirements and application instructions.

APPLICATION DEADLINE: All application materials must be electronically submitted or received at SFI no later than **January 27, 2006**.

For further information, please e-mail summerschool@santafe.edu or call +1 (505) 946-2746.

Women, minorities, and citizens of developing countries are especially encouraged to apply.

DIRECTOR OF MOLECULAR BIOLOGY & IMMUNOHISTOCHEMISTRY

Careers with Pennsylvania Hospital of the University of Pennsylvania Health System extend beyond the ordinary. Challenging and rewarding, they inspire achievement no matter what your discipline. Recognized as a leader by U.S. News & World Report, Pennsylvania Hospital is a 534-bed acute care unit facility located in the historic Society Hill section of Philadelphia, PA.

We are currently seeking a Director of Molecular Biology & Immunohistochemistry to provide administrative, technical, and general supervision of up to 5 staff members including molecular biology techs, immunohistochemistry techs, researchers and students. You will ensure compliance with institutional and laboratory policies and procedures, evaluate and recommend technical methods based on clinical and cost parameters, and ensure that written and approved technical procedures are maintained for each determination preformed.

A minimum of Ph.D. in Molecular Biology and extensive experience with molecular biology and IHC/ISH are required as is proven supervisory experience with the ability to interact effectively with all levels of Hospital personnel.

Enjoy a career with a history making health system. We offer an EXCELLENT salary and benefits package. For a complete position description and to apply online, please visit our Web site at www.pennhealth.com/jobs.

UNIVERSITY OF PENNSYLVANIA HEALTH SYSTEM
WE SEE LIFE AHEAD™

www.pennhealth.com/jobs

AA/EOE, M/F/D/V.

SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

Team Leader Positions
Mogam Biotechnology Research Institute

MBRI

Mogam Biotechnology Research Institute (MBRI) is an independent, non-profit organization and one of the leading research institutes in Korea in the field of biopharmaceutical development. The institute was founded in 1984 with the fund from the Green Cross Corp. (GCC), one of the top-rated pharmaceutical companies in Korea best known for its hepatitis B vaccine, and has been authorized as a WHO collaborating research center. In partnership with the GCC, we form a comprehensive R&D center, dedicated to applied research that extends from immunology, virology, and molecular biology, through drug discovery and development, to innovative human therapeutics and diagnostics.

Applications are invited for Team Leader Positions with a strong background in either:

- Immunotherapy and vaccines: Cellular immunology / Molecular virology
- Anti-cancer drugs: Cancer biology / Anti-angiogenesis
- Cell therapy: Self-renewal, differentiation and plasticity of stem cells
- Small chemical drugs: Medicinal chemistry
- Drug delivery: Gene, protein, and small molecule delivery
- Gene expression: Host cell engineering / Antibody engineering
- Large-scale process development: Experiences in industry

Salary and benefits are competitive and dependent on qualifications. Candidates must have a doctoral degree and strong research credentials. They will be expected to develop a strong and innovative research program that will ultimately lead to drug development.

Interested applicants should send their curriculum vitae and bibliography, and the names and addresses of three references to: Mogam Biotechnology Research Institute, 341 Bojeong-dong, Giheung-gu, Yongin 446-799, South Korea; E-mail: mogam@mogam.re.kr; Fax: +82-31-260-9808. Please be sure to indicate for which position the application is made.

Review will begin January 15, 2006, and continue until all positions are filled. For more information, applicants are encouraged to visit our website: www.mogam.re.kr

POSITIONS OPEN

FIELD STATION MANAGER
The University of Akron
Martin Center for Field Studies and Environmental Education

The Department of Biology is seeking a Field Station Manager for the newly acquired Martin Center for Field Studies and Environmental Education, located on the 400-acre Bath Nature Preserve. In addition to primary use by the Department of Biology, the preserve and field station are used by a variety of departments, including Geology, Geography and Planning, and Anthropology, for both research and environmental education classes. The successful applicant will have a Master's or Ph.D. degree in a field of environmental science or biology and will be responsible for oversight of field station building maintenance, including care and maintenance of research and teaching equipment; coordinating and participating in research/teaching activities at the station and on the nature preserve; and promoting development of the station according to its combined mission of teaching, research, and outreach. Preference will be given to candidates with proven teaching experience, especially in the area of biological and field/environmental studies. Candidates with previous experience managing or working in a field station are encouraged to apply. For further information visit the Department's **website: http://www.uakron.edu/biology/.**

Applicants should submit curriculum vitae, brief statements on research and teaching interests, and three letters of recommendation to: **Chair, Field Station Manager Search, Department of Biology, The University of Akron, Akron, OH 44325-3908.** *The University of Akron is committed to a policy of Equal Employment Opportunity and to the principles of Affirmative Action in accordance with state and federal laws.*

MEDICAL MICROBIOLOGY FACULTY (nine-month, tenure-track), Ferris State University. Teach introductory and advanced courses in medical microbiology and immunology to allied health and preprofessional undergraduates and to doctoral students in the College of Pharmacy and the Michigan College of Optometry. Additional responsibilities: student advising, curriculum development, and continuing professional development. Required: Ph.D. in medical microbiology and a strong commitment and potential for teaching excellence in both lecture and laboratory and interpersonal and communication skills to be able to work effectively with a diverse array of students and colleagues. Preferred: At least one year full-time teaching at the college or university level and an interest in implementing a research program that will involve undergraduates. Review of applications will begin December 1, 2005, and continue until the position is filled. Submit letter of application clearly stating qualifications for position, curriculum vitae, statement of teaching philosophy, unofficial copies of all college transcripts (both undergraduate and graduate), and names, addresses, telephone numbers, and e-mail addresses of three references to: **James D. Hoerter, Ph.D., Department Head, Biological Sciences, 820 Campus Drive, ASC 2004, Big Rapids, MI 49307.** For more information about Ferris State University, please visit our **website: http://www.ferris.edu/.** *An Equal Opportunity/Affirmative Action Employer.*

THE COLLEGE OF WOOSTER

BIOLOGIST. One-year position for 2006-2007 with ability to teach introductory biology and two upper-level courses in ecology, evolution, and/or conservation biology at The College of Wooster in Wooster, Ohio. See description at **website: http://www.ohio5.org/faculty.htm.** Choose Biology to locate the description or contact **Dr. Dean Fraga,** Chair, Department of Biology at **telephone: 330-263-2557** or **e-mail: dfraga@wooster.edu.** *The College of Wooster is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

The Department of Biological Sciences at Marshall University announces a tenure-track position at the **ASSISTANT PROFESSOR** level to begin fall 2006. We seek a Watershed Biologist with expertise in limnology, microbial ecology, aquatic toxicology, ichthyology, or other related discipline. Responsibilities include teaching in the introductory majors' biology series and development of an upper-level/graduate course in the candidate's specialty area. Candidates with an interest in interdisciplinary teaching and research are especially encouraged to apply. Job requirements also include mentoring students, establishing an extramurally funded research program using modern quantitative research methods, and participating in Department and University committees. Candidates must possess a Ph.D. in biology or a closely related discipline. Those with demonstrated excellence in undergraduate teaching, experience working with undergraduates in research, and postdoctoral research experience will be given special consideration. Qualified candidates should send a cover letter, curriculum vitae, graduate transcripts, statement of teaching goals, statement of research interests and goals, selected reprints, and three letters of reference (sent directly to the address below). All application materials should be addressed to: **Dr. Charles Somerville, Chair, Department of Biological Sciences, Watershed Biologist Search, Marshall University, One John Marshall Drive, Huntington, WV 25755.** Review of completed applications begins on December 15, 2005, and will continue until the position is filled. Information about Marshall University and Huntington, West Virginia, can be found at **websites: http://www.marshall.edu** and **www.hadco.org.** *Marshall University is an Affirmative Action/Equal Employment Opportunity Employer and encourages applications from women, minorities, and persons with disabilities.*

BIOCHEMISTRY LABORATORY INSTRUCTOR, University of Maryland, Baltimore County. Full-time, nontenure-track position involving teaching undergraduate biochemistry laboratory course in a research-active environment, as well as other teaching at the introductory level. The laboratory course surveys basic biochemical methods. The instructor is responsible for both lecture and laboratory components, including supervision of teaching assistants. He/she will take an active role in ongoing course development. M.S. required, Ph.D. preferred. Position available August 2006. Send resume and arrange for three letters of reference to be sent to: **Dr. Richard L. Karpel, Chair, Biochemistry Laboratory Instructor Search, Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250. E-mail: karpel@umbc.edu.** Applicants will be considered until the position is filled. *UMBC is an Equal Opportunity/Affirmative Action Employer. Minorities, Women, and Individuals with Disabilities are encouraged to apply.*

TENURE-TRACK POSITION
MARINE CHEMISTRY

Applications are invited for a Tenure-Track position in the Department of Chemistry and Biochemistry at Florida International University in the broadly defined area of marine chemistry with appointments starting in fall 2006. A Ph.D. in chemistry/marine chemistry and postdoctoral experience are required. The position is in support of developing the Marine Sciences Program and is based at Biscayne Bay Campus. Candidates are required to establish an active, externally funded research program directing Ph.D., M.S., and undergraduate students. Please see **website: http://www.fiu.edu/orgs/chemistry** for more details. Send curriculum vitae, transcripts, research plans, and three letters of reference to**: Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199.** Selection process will begin January 31, 2006. *FIU is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

TENURE-TRACK PROFESSORS
Watershed Systems, Marine Science and Policy

The Division of Science and Environmental Policy at California State University, Monterey Bay invites applications for tenure-track **ASSISTANT PROFESSORS** in watershed systems and marine science and policy to begin August 2006. Successful applicants will contribute excellence in teaching, learning, and applied research within the B.S. program in Earth Systems Science and Policy and an M.S. program in Coastal and Watershed Science and Policy. We seek applicants whose work is relevant to environmental policy or resources issues.

Marine science and policy applicants should demonstrate interdisciplinary excellence in some subset of the following fields: marine science, geographic information systems/global positioning system, remote sensing, modeling, oceanographic instrumentation, marine policy, and economics. Preference will be given to applicants who can infuse policy into their marine science teaching and research and are familiar with issues impacting coastal California.

Watershed systems applicants should demonstrate interdisciplinary excellence in some subset of the following watershed fields: hydrology/ecology/restoration, chemical/biological water quality, river/riparian/lagoon ecosystems, or remote sensing. Preference will be given to applicants who can use GIS in teaching and research and are familiar with watershed issues impacting coastal California.

A Ph.D. is required at the time of hiring. Salary is commensurate with experience. To apply or to review a more detailed position description visit **website: http://uhr.csumb.edu/jobs/db/fac/.** Applications received before 5:00 p.m. January 6, 2006, are ensured a full review. Open until filled.

The University of Florida McKnight Brain Institute (MBI), UF Shands Cancer Center (UFSCC), the Department of Neurological Surgery, and the Department of Pharmacology and Therapeutics in the College of Medicine are expanding the neuro-oncology program with a focus on molecular therapeutics. Positions are open for tenure-track neuro-oncology researchers having a Ph.D. and/or M.D. degree, at the **ASSISTANT/ASSOCIATE/FULL PROFESSOR** levels. We are especially interested in the fields of cancer stem cell biology, immunology, and cell signaling with an eye toward the development of new cellular and molecular therapeutic approaches for brain cancer. Substantial startup packages and endowments will support the investigators to help build a program dedicated to the development of new therapeutics for invasive brain tumors. Additional information about the research and educational programs in the MBI, UFSCC, and the Department are available at **websites: http://www.mbi.ufl.edu, http://www.ufscc.ufl.edu,** and **http://www.med.ufl.edu/pharm,** respectively. Applications consisting of curriculum vitae and three letters of recommendation will be accepted until December 31, 2005. Please contact: **Dr. Dennis A. Steindler, Executive Director, The Evelyn F. and William L. McKnight Brain Institute of the University of Florida, College of Medicine, P.O. Box 100015, Gainesville, FL 32610. E-mail: steindler@mbi.ufl.edu.** *This is an Equal Opportunity Institute.*

FACULTY POSITIONS
PONDICHERRY UNIVERSITY
PONDICHERRY-605014, I

Pondicherry University, a federally funded multifaculty University, is seeking well-qualified, highly motivated and experienced Indian nationals to join as faculty members in physics (Professor-1, Reader-1 and Lecturers-3), chemistry (Professor-1, Reader-1), biochemistry and molecular biology (Professor/Reader-1, Lecturers-2), biotechnology (Lecturer-1), mathematics (Professor-1), statistics (Professor-1), and Earth sciences (Lecturer-1). The interested candidates may visit the University **website: http://www.pondiuni.org** for further details and send their academic vita addressed to the vice-chancellor by post or by **e-mail: pcu_vc@yahoo.co.in.**

10yr Anniversary

جامعة كارنيجي ميلون قطر
Carnegie Mellon QATAR CAMPUS

Qatar Foundation

Human-Computer Interaction Visiting Faculty Position in the School of Computer Science

Carnegie Mellon University established a branch campus in Qatar in the fall of 2004. We are offering a BS degree in Computer Science to an international student body. The university invites applications for a visiting faculty position to begin as early as January 2006.

We are seeking a faculty member in the area of Learning Science and Technology with research experience ideally in designing, implementing, deploying, and evaluating educational technology in school or college settings. An ability to teach courses in human-computer interaction, artificial intelligence, cognitive psychology, or related areas is also desired. The position will involve research in collaboration with the Pittsburgh Science of Learning Center and faculty at the Human-Computer Interaction Institute at Carnegie Mellon in Pittsburgh. The position offers competitive salaries, overseas assignments, travel and housing allowances and other benefits packages, as well as attractive research support.

Interested candidates should send their resume, statement of teaching interest and research, and names of three references to: **Faculty Hiring Committee, c/o Ruth Gaus, Qatar Office SMC 1070, 5032 Forbes Avenue, Pittsburgh, PA 15289; Ruth.Gaus@cs.cmu.edu; Fax 412-253-0924.**

- For more information on the Pittsburgh Science of Learning Center, see **http://learnlab.org**.
- For more information on the Human-Computer Interaction Institute, see **http://www.hcii.cs.cmu.edu**.
- For more information on the BS in CS program, see **http://www.csd.cs.cmu.edu/education/bscs/index.html**.
- For more information on the Carnegie Mellon Qatar Campus, see **http://www.qatar.cmu.edu/.**
- Information on Qatar is available at: **http://www.experienceqatar.com/** .

MGH 1811 MASSACHUSETTS GENERAL HOSPITAL

Senior Investigator - Brain Tumor Research

The Massachusetts General Hospital is seeking an independent, senior scientist to establish a dynamic, integrative research program in the biology of gliomas. Potential areas of interest include mouse models, tumor stem cell biology, pharmacology, growth factors and immunology although other themes will be considered. The appropriate candidate should hold either a Ph.D. or M.D. degree or both. Departmental assignment within Harvard Medical School will depend on research focus. The candidate should be an accomplished investigator as demonstrated by peer-reviewed grant support and publications in the biomedical literature. The appropriate candidate should be able to develop concepts that lead to a better understanding of the biology of gliomas and their therapy.

This opportunity includes generous start-up funds and new laboratory space in the Simches Research Center at MGH that opened in 2005. The position features many opportunities to collaborate with other scientists involved in glioma research at Massachusetts General Hospital. This position will also include collaboration with a large clinical brain tumor program at Harvard Medical School supported by multiple NIH grants and the opportunity to supervise research fellows supported by an NCI-sponsored training grant in neuro-oncology. Applications from women and representatives of minority groups are encouraged. Interested candidates should forward a curriculum vitae, a brief statement of research interests and 3 letters of recommendation to: **Valerie J. Smith; Stephen E. and Catherine Pappas Center for Neuro-Oncology; Yawkey 9E; Massachusetts General Hospital; 55 Fruit Street; Boston, MA 02114-2696; vjsmith@partners.org**.

The Massachusetts General Hospital is an Equal Opportunity Employer.

WPI

FACULTY POSITIONS

ASSISTANT/ASSOCIATE PROFESSOR BIOLOGY & BIOTECHNOLOGY

The Department of Biology & Biotechnology invites applications for tenure-track, academic year faculty positions at the established ASSISTANT or ASSOCIATE PROFESSOR level. These positions will complement existing or contribute to targeted strengths in cell, molecular, and developmental/regenerative biology, ecology/evolution, and computational biology. These positions are part of a strategic initiative that includes a new WPI Life Science and Bioengineering Center, under construction. Research areas of particular interest include **Bioinformatics, Molecular Ecology/Evolution, and Stem Cell/Regenerative Biology**, in plant or animal systems. Individuals that employ computational research approaches are especially sought. Applicants will possess the Ph.D. degree and are expected to have established vigorous, extramurally funded research programs in their area of expertise, as well as a strong teaching record at the undergraduate and/or graduate level. Compensation, new laboratory space and resources for startup funding are very competitive and commensurate with research experience and accomplishments.

WPI is a nationally ranked, selective technology-centered university with 2,900 undergraduate and over 1,000 graduate students. The Department of Biology & Biotechnology currently enrolls ~225 undergraduate majors and ~20 graduate students (MS & Ph.D.). Worcester, New England's third largest city, and the surrounding area offer diverse academic, economic, cultural, and recreational resources. Opportunities for collaboration exist among several neighboring institutions including the University of Massachusetts Medical School and Tufts Cummings School of Veterinary Medicine.

Interested candidates should send a curriculum vitae, description of research plan, a statement of teaching philosophy, and a list of five references (with full contact information) to: **Biology & Biotechnology Search Committee, Worcester Polytechnic Institute, Human Resources, 100 Institute Rd., Worcester, MA 01609-2280.** You may email your information to: **human-resources@wpi.edu.** NO PHONE CALLS PLEASE. Review of applications will begin on **December 1, 2005** and continue until the position is filled. For additional information and inquiries about the department and WPI visit: **http://www.wpi.edu/Academics/Depts/Bio/**

WPI offers a smoke free environment. To enrich education through diversity, WPI is an affirmative action, equal opportunity employer. Women and minorities are especially encouraged to apply.

ASSOCIATE TOXICOLOGIST
STAFF TOXICOLOGIST
SENIOR TOXICOLOGIST

The Department of Pesticide Regulation in the California Environmental Protection Agency announces recruitment for the positions of Senior Toxicologist, Staff Toxicologist, and Associate Toxicologist, for the purpose of establishing hiring lists. Openings exist in Sacramento, California. Persons experienced in conducting human health pesticide exposure assessments, or evaluating toxicological or exposure studies are encouraged to apply. To qualify for Associate Toxicologist, an entry level position, persons must: (1) hold a doctoral degree in toxicology or a closely related field (e.g., public health, bio- or environmental chemistry, pharmacology, physiology) from an accredited university; or (2) hold a master's degree in toxicology or a closely related field from an accredited college or university and three years post-master's experience performing and interpreting studies and/or conducting safety evaluations; or (3) have certification as a Diplomat of the American Board of Toxicology. To qualify for Staff Toxicologist, persons must hold a doctoral degree in toxicology or a closely related field and a minimum of 3 years of relevant post-doctoral experience, which must include interpretation of toxicological findings relative to probable human health or environmental hazards, and 1 year of experience developing and designing toxicological research and investigative studies. Persons in the position of Senior Toxicologist will supervise a group of toxicologists and perform the most difficult evaluations and assessments related to human health. Persons qualified for this position must hold a doctoral degree in toxicology or a closely related field and a minimum of 4 years relevant post-doctoral experience, which must include the interpretation of toxicological findings relative to probable human health or environmental hazards, and 1 year of experience developing and designing toxicological research and investigative studies. The salary ranges for Associate, Staff, and Senior Toxicologist are **$4,516-$5,984, $5,984-$7,239**, and **$6,284-$7,597** per month, respectively. At a later date, you will be sent a supplemental application to complete. If interested, please submit your resume to the following: **Department of Pesticide Regulation, Medical Toxicology Branch, Attention: Dr. Gary Patterson, 1001 "I" Street, P.O. Box 4015, Sacramento, CA 95814-4015.** Please submit your resume no later than **December 31, 2005**.

POSITIONS OPEN

ASSISTANT PROFESSOR OF ENVIRONMENTAL SCIENCE: ENVIRONMENTAL HEALTH
Tenure Track, Commencing Fall 2006

Description: Will teach introductory environmental science for majors and nonmajors, environmental/occupational health for nursing students, and develop electives in area of expertise. The successful candidate will be able to do both field and statistical research, be adept in working in an interdisciplinary environment, and have a global perspective. A working knowledge of geographic information systems is a plus. Responsibilities include teaching day and evening courses, advising students, engaging in scholarship/professional development, and participation in faculty governance.

Requirements: Ph.D. in biology, environmental health, environmental science, or other applicable area. All but dissertation with imminent completion date will be considered, but all requirements for a Ph.D. must be completed one month prior to the appointment. Must have applied experience related to environmental health or public health. Teaching experience at undergraduate level preferred.

Faculty members are expected to maintain active participation in research, scholarship, college governance, service, academic advisement, and professional development activities.

All applications must be completed online at **website: http://www.ramapojobs.com.** Attach resume, cover letter, statement of teaching philosophy and research interests, and a list of three references to your completed application. Since its beginning, Ramapo College has had an intercultural/international mission. Please tell us how your background, interest, and experience can contribute to this mission as well as to the specific position for which you are applying.

Review of applications will begin immediately and continue until the position is filled. Position offers excellent state benefits. To request accommodations, **telephone: 201-684-7734.**

Dr. Eric Karlin, Search Committee Chair
Ramapo College of New Jersey
Department 25, 505 Ramapo Valley Road
Mahwah, NJ 07430

Ramapo College is a member of the Council of Public Liberal Arts Colleges, a national alliance of leading liberal arts colleges in the public sector.

Ramapo College of New Jersey is located in the beautiful foothills of the Ramapo Valley Mountains approximately 25 miles northwest of New York City. Accredited by the Middle States Commission on Higher Education, Ramapo College is a comprehensive institution of higher education dedicated to the promotion of teaching and learning within a strong liberal arts–based curriculum, thus earning the designation "New Jersey's Public Liberal Arts College." Its curricular emphasis includes the liberal arts and sciences, social sciences, fine and performing arts, and the professional programs within a residential and sustainable living and learning environment. Organized into thematic learning communities, Ramapo College provides academic excellence through its interdisciplinary curriculum, international education, intercultural understanding, and experiential learning opportunities.

Equal Employment Opportunity/Affirmative Action.

POSTDOCTORAL SCIENTISTS and RESEARCH ASSOCIATE SCIENTISTS
Molecular Basis of Cancer

Positions available to characterize novel genes mediating tumorigenesis, cancer progression and suppression, apoptosis, differentiation, and senescence. Training in molecular biology and biochemistry required. Experience with transgenic mice, protein analysis, and purification or protein-protein interactions desirable. Send curriculum vitae with reference list to: **Dr. Paul B. Fisher, Columbia University Medical Center, College of Physicians and Surgeons, BB 15-1501, 630 West 168th Street, New York, NY 10032. E-mail: pbf1@columbia.edu.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

ASSISTANT PROFESSOR: MAMMALIAN DEVELOPMENTAL GENETICS. The Biochemistry and Cellular and Molecular Biology (BCMB) Department at the University of Tennessee seeks to fill a tenure-track faculty position at the Assistant Professor level to begin in August 2006. We will particularly welcome applications from individuals who apply genomic or proteomic methods and/or use mouse genetic models to address problems in developmental biology, and from individuals with interests in developmental neurobiology, but outstanding applications from individuals in all areas of developmental genetics will be considered. The successful candidate for this position will benefit from interactions with strong research groups within the BCMB Department and in other units on campus and at the nearby Oak Ridge National Laboratory in neurobiology, chromatin and chromosome dynamics, biology of cancer and aging, cell division and cell cycle, structural biology, enzyme mechanisms, mouse genetics/genomics, proteomics, and computational biology. The successful applicant will be expected to develop an independent, externally funded research program in mammalian developmental genetics, to provide state-of-the-art training for graduate students and postdoctoral researchers, and to contribute to the teaching mission of the BCMB Department at both the undergraduate and graduate levels. Required qualifications include a Ph.D. and postdoctoral experience in relevant areas of biology, evidence of significant scientific productivity, and a commitment to an integrated program of teaching and research. The University welcomes and honors people of all races, creeds, cultures, and sexual orientations, and values intellectual curiosity, pursuit of knowledge, and academic freedom and integrity.

Interested candidates should send a cover letter, a resume, a description of research experience and of the proposed research program, and the names of three individuals who can provide letters of reference to: **Ranjan Ganguly, Chair, Faculty Search Committee, Biochemistry and Cellular and Molecular Biology Department, M407 WLS, University of Tennessee, Knoxville, TN 37996-0840.** Review of applications will begin on December 1, 2005, and continue until the position is filled.

The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA Institution in the provision of its education and employment programs and services.

The Division of Endocrinology and Diabetes in the Department of Medicine at the University of Southern California Keck School of Medicine is seeking applications for two tenured or tenure-track **FACULTY POSITIONS** to complement strong existing programs in translational and clinical research in type 2 diabetes and obesity. Applicants must hold M.D. with or without Ph.D. and should either have a successful basic research program or demonstrate strong potential to develop such a program. Applicants should send a letter of interest and current curriculum vitae to: **Thomas A. Buchanan, M.D., Chief, Division of Endocrinology and Diabetes, University of Southern California Keck School of Medicine, Room 6602 General Hospital, 1200 N. State Street, Los Angeles, CA 90033.** *The University of Southern California is an Equal Opportunity Employer.*

One **POSTDOCTORAL POSITION** is available immediately to study the role of tissue factor pathway inhibitor-2 in extracellular matrix homeostasis. Applicants must have a recent Ph.D. degree in biochemistry or related field with expertise in molecular biology. Experience in protein isolation and characterization is also desirable. Send curriculum vitae and the names of three references to: **Dr. Walter Kisiel, Department of Pathology, University of New Mexico School of Medicine, MSC 08-4640, Albuquerque, NM 87131. Fax: 505-272-5139; e-mail: wkisiel@salud.unm.edu.** *The University of New Mexico is an Affirmative Action/Equal Opportunity Institution.*

Microbiologist: ASSISTANT PROFESSOR, tenure-track, Ph.D. required. Position will begin in September 2006. The candidate is expected to supervise undergraduate research and to teach introductory biology classes, introduction to research, and microbiology. Preference will be given to candidates who have the background and ability necessary to initiate a course in genomics. Depending upon the successful candidate's background, he/she may also be asked to teach upper-level courses in gene expression, plant physiology, or molecular biology. The candidate will have the opportunity to develop courses in his/her specialty and will be expected to develop a research program accessible to undergraduates as well as maintain his/her own research. Department facilities include a tissue culture laboratory, electron microscope laboratory, an imaging facility, an animal museum, herbarium, greenhouse, aquarium room, and access to a 289-hectare biological field station. Candidates should submit curriculum vitae, statements of teaching philosophy and research interests, and three letters of recommendation to: **Dr. Stuart Allison, Biology Department, Knox College, Galesburg, IL 61401.** We will start reviewing applications on December 15, 2005, and continue until the position is filled. For more information about biology at Knox check **website: http://www.knox.edu/biology.xml.** *In keeping with its 169-year commitment to equal rights, Knox College particularly welcomes applications from women and members of other historically underrepresented groups.*

POSITION AVAILABLE
Mass Spectrometry and Protein Analysis
University of Vermont
Department of Biology

Applications are invited for a **RESEARCH FACULTY** member in the Department of Biology in the area of mass spectrometry and protein analysis. This faculty member will provide specialized technical expertise for a mass spectrometry facility supported by the Vermont Genetics Network. Assist users in design and conduct of experiments using mass spectrometry, use mass spectrometers to run samples for users, provide data analysis, and perform equipment maintenance.

All applicants are expected to hold an M.S. or Ph.D. degree in a field related to biological mass spectrometry and experience with mass spectrometry instrumentation and protein analysis. Effective oral and written communication skills are a must.

Candidates should apply online at **website: http://www.uvmjobs.com** and must attach to that application curriculum vitae, a statement of interest in working with mass spectrometry instruments, and names with contact information of three references.

The University of Vermont is an Affirmative Action/Equal Opportunity Employer. The Department is committed to increasing faculty diversity and welcomes applications from women and underrepresented ethnic, racial, and cultural groups and from people with disabilities.

PROFESSOR AND HEAD
Biological Sciences Department
North Dakota State University

Applicants are sought for leadership of a department with 15 faculty members; B.S., M.S., Ph.D. programs; and a current research budget greater than $1 million per year (**website: http://biology.ndsu.nodak.edu**). Focal areas include: (1) ecology, evolution, and conservation biology, (2) developmental and regulatory biology, (3) science education. Requirements include a Ph.D. in a relevant area, an established research program, evidence of administrative capabilities, and academic experience appropriate to rank of Professor. Submit a cover letter, curriculum vitae, description of research, representative reprints, statement of leadership philosophy, and a list of at least three references to: **Department Head Search Committee, Biological Sciences, North Dakota State University, Fargo, ND 58105-5517.** Review of applications will begin December 19, 2005, and continue until position is filled. *NDSU is an Equal Opportunity Institution.*

PROFESSOR AND CHAIR
HARVARD MEDICAL SCHOOL
DEPARTMENT OF GENETICS

Harvard Medical School is seeking an academic and scientific leader to fill the position of Chair, Department of Genetics. The candidate should be an exceptional geneticist and a visionary leader who thinks broadly about genetics, with a general understanding and appreciation for the full spectrum of genetics research, from cell culture models, to model organisms, to human genetics. The Chair should also be an exemplary teacher, dedicated to medical and graduate education, and the mentoring of junior faculty. The candidate should be generous in spirit and highly-motivated to lead the department and to collaborate with leaders in the Boston community to integrate research and educational activities within the broad missions of the Department and the Harvard Medical School. Experience in coordinating and administering complex research and/or educational activities is preferred. Interested individuals should send a letter of application and current CV in hard copy and electronic format to:

Joan S. Brugge
Chair, Department Cell Biology
Harvard Medical School
240 Longwood Avenue, Boston, MA 02114
E-mail: Joan_Brugge@hms.harvard.edu

Harvard Medical School is an equal opportunity/affirmative action employer with a strong institutional commitment to diversity. Women and minority candidates are particularly encouraged to apply.

HARVARD MEDICAL SCHOOL

UNIVERSITY OF
MASSACHUSETTS LOWELL

Tenure-Track Position
Biological Sciences

The University of Massachusetts Lowell Department of Biological Sciences invites applications for a tenure-track position, rank negotiable, to start in Fall 2006. The successful candidate will be expected to build a vigorous, externally funded research program, and collaboration within this and other departments is encouraged. Current faculty research interests include bioinformatics, genetics, plant science, neurobiology, cancer biology, invertebrate biology, developmental biology, virology, microbial ecology and biogeochemistry. Our campus is located very near the vibrant academic and commercial biotechnology centers of Boston, Cambridge and Worcester. We are seeking individuals with expertise in one or more of the following areas: Genetics, Population Genetics, Evolution, and/or Conservation Biology. Teaching obligations include development of upper-level undergraduate/graduate courses in her/his expertise, and participation in the teaching of core undergraduate courses as needed. A curriculum vita, copies of several recent research publications, a statement of research, teaching interests not to exceed three pages, and arrangement for three letters of recommendation should all be sent to the search committee. Apply by **December 24, 2005,** (hard copy only) to: **Search Committee for Assistant/Associate Professor in Biological Sciences, C/O Dr. Brian Bettencourt, University of Massachusetts Lowell, Department of Biological Sciences, One University Avenue, Lowell, MA 01854.**

University of Massachusetts Lowell
UMASS Lowell

The University of Massachusetts is an Equal Opportunity/Affirmative Action Title IX, H/V, ADA 1990 Employer and Executive Order 11246, 41 CFR60-741 4, 41 CRF60-250 4, 41CRF60-1 40 and 41 CFR60-1, 4 are hereby incorporated.

Faculty Position

Division of Genomic Stability and DNA Repair

Dana-Farber Cancer Institute

Applications are invited for a tenure track position as Assistant Professor at Harvard Medical School, in the Department of Biological Chemistry and Molecular Pharmacology. The appointee will join a superb faculty, dedicated to the study of genomic stability, DNA repair, and the DNA damage response. Applicants (M.D. or Ph.D.) with outstanding research experience in related fields (biochemistry, DNA repair, checkpoint biology, genetics) are especially encouraged to apply.

Candidates should send curriculum vitae, three references, and a description of future research to:

Alan D'Andrea, M.D.
Chief, Division of Genomic Stability and DNA Repair
Dana-Farber Cancer Institute
44 Binney Street
Boston, MA 02115

Qualified women and minority candidates are encouraged to apply.

MIAMI UNIVERSITY

Professor and Chair of Microbiology

Applications and nominations are invited for Professor and Chair of the Department of Microbiology on the Oxford, Ohio campus, beginning by August 2006. The University is seeking an outstanding senior scholar with an excellent record in research and effective teaching to provide leadership that would complement and expand the department's strengths in research and in undergraduate and graduate education.

Miami University is ranked among the top national public universities. The Oxford campus, near Cincinnati, has over 16,000 students. The Department offers undergraduate and advanced graduate degrees and participates in interdisciplinary graduate programs in Molecular Biology and Ecology, and the MAT in Biological Science. The Department is committed to educational and scholarly excellence, faculty/student diversity, and international preeminence. The Electron Microscopy Facility, Center for Bioinformatics and Functional Genomics, Ecological Research Center, and Animal Facilities offer research support.

www.cas.muohio.edu/micro

Applicants must submit a cv; brief essays on leadership goals and vision including evidence of administrative ability and/or potential, on research interests and goals, and on teaching philosophy; 3 reprints; and contact information for 5 references. Exceptional candidates at the Associate Professor level will also be considered.

Send applications to Dept. of Microbiology, attn. Chair Search, 32 Pearson Hall, Miami University, Oxford, OH 45056; or by email to microbiology@muohio.edu (single pdf plus reprints). Screening begins 1 February 2006 and will continue until position is filled.

Miami University is an equal opportunity affirmative action employer.

FACULTY POSITIONS
DIVISION OF MEDICINAL CHEMISTRY AND NATURAL PRODUCTS
THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
SCHOOL OF PHARMACY

The Division of Medicinal Chemistry and Natural Products invites applications for the faculty position at the Assistant or Associate Professor level; exceptional candidates at the Full Professor level will be considered as well. Applicants with research interests in all areas of medicinal chemistry and chemical biology will be considered, but preference will be given to programs that emphasize the development of small molecules for use in the study of protein and cell functions, particularly at the genome level, and neurological and other pathways relevant to physiology and disease. The candidate should have an externally funded and internationally recognized record of research and scholarship, commensurate with rank. The position requires the demonstration of excellence in graduate and professional education. Applications will be reviewed upon receipt and continue until the positions are filled. Applications must include the following materials: curriculum vitae, publication list, a concise description of past research and future research plans, and four letters of recommendation sent directly to the search committee, on your behalf. Contact: **Prof. Alexander Tropsha, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, CB #7360, Beard Hall, Chapel Hill, NC 27599-7360.** *The University of North Carolina at Chapel Hill is an Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.*

POSITIONS OPEN

Washington University in St. Louis
SCHOOL OF MEDICINE

FACULTY POSITION
Mechanistic Enzymology/Single Molecule Studies

The Department of Biochemistry and Molecular Biophysics at Washington University School of Medicine invites applications for a tenure-track or tenured faculty position with a research focus in the area of mechanistic enzymology. Applicants at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level will be considered. The successful candidate will conduct independent research within a growing Department that is broadly represented in quantitative studies of macromolecules, including enzymology, molecular interactions and dynamics, structural biology, and computation (**website: http://www.biochem.wustl.edu**). Innovative methods for the visualization and functional analysis of single molecules are of particular interest, although not a requirement. Enthusiasm for teaching and mentoring young scientists is important.

Washington University has a highly interactive research environment with vigorous interdisciplinary graduate and medical scientist training programs. Selection of candidates will begin in December 2005. Applicants should submit their curriculum vitae, selected reprints, a short summary of future research plans and the names of references electronically to **e-mail: enzympos@biochem.wustl.edu,** or else by mail to:

Enzymology Search
Tom Ellenberger, Raymond H. Wittcoff
Professor and Head
Department of Biochemistry and Molecular
Biophysics, Box 8231
Washington University
School of Medicine
660 S. Euclid Avenue
St. Louis, MO 63110

An Equal Opportunity Employer. Minority and women scientists are especially encouraged to apply.

ASSISTANT/ASSOCIATE/FULL PROFESSOR
Pharmaceutics and Medicinal Chemistry

Temple University School of Pharmacy invites applications for two tenure-track faculty positions at Assistant, Associate, or Full Professor level in the Department of Pharmaceutical Sciences. Faculty will be expected to maintain an independent research program and to contribute to teaching professional and graduate students. Qualified applicants in pharmaceutics and medicinal chemistry are especially encouraged to apply. The university is in the midst of unprecedented growth and offers many research opportunities.

Review of applications will begin immediately and continue until the positions are filled. Interested applicants should mail a letter of intent, curriculum vitae, and the contact information for three references by regular mail or e-mail to: **Ellen A. Walker, Ph.D., Chair, Search Committee, Temple University School of Pharmacy, 3307 North Broad Street, Philadelphia, PA 19140. E-mail: ellen.walker@temple.edu.**

Temple University is an Equal Opportunity/Affirmative Action/ADA Employer.

Choose
TEMPLE UNIVERSITY

POSTDOCTORAL POSITION
NEUROPHARMACOLOGY

Immediate opening for a position that will combine substance abuse research using a rat model with half-time support of our animal research facility. Experience with animal surgery, microdialysis, and neuroanatomy techniques preferred. Send research interests, curriculum vitae, and references to: **Kenneth Grasing, M.D., Associate Chief of Staff for Research, Kansas City VA Medical Center, 4801 E. Linwood Boulevard, Kansas City, MO 64128. E-mail: kenneth.grasing@va.gov.**

POSITIONS OPEN

The Department of Biological Sciences at Marshall University invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level beginning fall 2006. Qualifications: A Ph.D. in biology or a related discipline is required and postdoctoral experience is preferred. The successful candidate will be expected to seek external research funds. Research fields that would complement existing strengths include comparative/functional morphology, biosystematics, or vertebrate paleobiology. Primary teaching responsibilities will include introductory human anatomy. Additionally, the opportunity will exist to develop an upper-level course in comparative/functional morphology or biosystematics. Applicants must have a sincere interest in, and commitment to, undergraduate education and research. Startup package and laboratory space negotiable depending upon experience. Please submit a letter of application, curriculum vitae, a one- to two-page statement of teaching philosophy and research goals, copies of graduate transcripts, and names, e-mail addresses, and telephone numbers of three references to: **Anatomy Search Committee, Department of Biological Sciences, Marshall University, One John Marshall Drive, Huntington, WV 25755.** Electronic submission in the form of a PDF document is preferred at **e-mail: straitho@marshall.edu.** To learn more about the Department of Biological Sciences at Marshall University, visit **website: http://www.marshall.edu/biology/.** Review of applications will begin December 15, 2005, and continue until the position is filled. *Marshall University is an Affirmative Action/Equal Employment Opportunity Employer.*

TENURE-TRACK FACULTY POSITION
PHYSIOLOGY

The Department of Zoology seeks applicants for a tenure-track **ASSISTANT PROFESSOR** in physiology to begin in August 2006. The successful candidate will have a Ph.D. in physiology or a related field and will be expected to develop a strong, independent and externally funded research program, supervise research by undergraduate, M.S., and Ph.D. students, and teach graduate and undergraduate courses. Preference will be given to applicants with postdoctoral experience and expertise that complements existing research strengths in the Department (see **website: http://zoology.muohio.edu/**). Send letter of application, curriculum vitae, statement of teaching and research interests, and three letters of recommendation to: **Dr. Douglas Meikle, Chair, Department of Zoology, Miami University, Oxford, OH 45056. Telephone 513-529-3100; e-mail: meikled@muohio.edu** for more information. Review of applicants will begin on 15 December 2005 and continue until the position is filled. *Women and minority candidates are encouraged to apply. Miami University offers Equal Opportunity in Employment and Education.*

POSTDOCTORAL POSITION

An NIH-funded Postdoctoral position is available immediately to study molecular mechanisms of differential body and organ growth. The research is directed toward molecular mechanisms that govern phenotypic evolution in selected arthropod groups, and our current focus is on genetics of leg size variation in insects (*PNAS* **101:**4877-4882, 2004). Applicants with expertise and interests in developmental genetics, insect biology, and invertebrate/insect transgenics will be given special consideration. Prospective candidates should send a letter indicating research interests, curriculum vitae, and contact information for three references to:

Dr. Aleksandar Popadic
Department of Biological Sciences
Wayne State University
5047 Gullen Mall
Detroit, MI 48202
E-mail: apopadic@biology.biosci.wayne.edu.

POSITIONS OPEN

Washington University in St. Louis
SCHOOL OF MEDICINE

FACULTY POSITION
Structural Biology

The Department of Biochemistry and Molecular Biophysics at Washington University School of Medicine invites applications for a tenure-track or tenured faculty position in the area of structural biology. Applicants at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level will be considered. The successful candidate will conduct independent research within a growing Department that is broadly represented in quantitative studies of macromolecules, including X-ray and nuclear magnetic resonance studies of protein structure and dynamics, macromolecular interactions, mechanistic enzymology, and computational analyses and modeling (**website: http://www.biochem.wustl.edu**). Priority will be given to candidates using integrated approaches to the study of macromolecular structure and biological function(s). Enthusiasm for teaching and mentoring research trainees is important.

Washington University has a highly interactive research environment with vigorous interdisciplinary graduate and medical scientist training programs. Selection of candidates will begin in December 2005. Applicants should submit their curriculum vitae, selected reprints, a short summary of future research plans, and the names of references electronically to **e-mail: structurepos@biochem.wustl.edu** or else by mail to:

Structural Biology Search
Tom Ellenberger, Raymond H. Wittcoff
Professor and Head
Department of Biochemistry and Molecular
Biophysics, Box 8231
Washington University
School of Medicine
660 S. Euclid Avenue
St. Louis, MO 63110

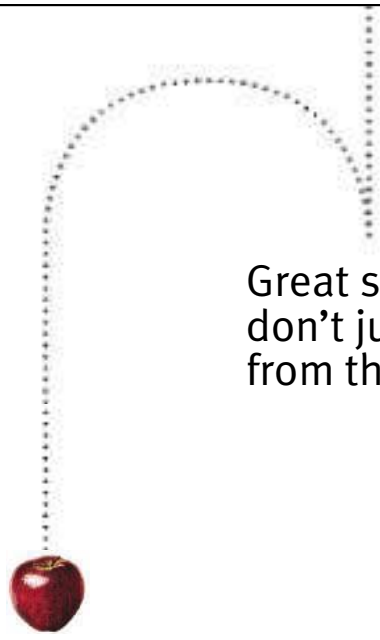
An Equal Opportunity Employer. Minority and women scientists are especially encouraged to apply.

POSTDOCTORAL RESEARCH FELLOW

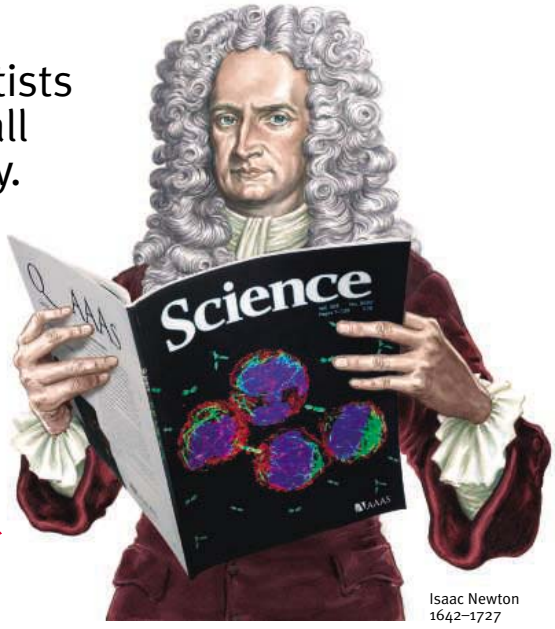
A Postdoctoral Position is available immediately in the laboratory of **Dr. Joanna Davies**, in the field of immune regulation. Applicants should have a Ph.D. in immunology with an emphasis in cellular immunology, and be familiar with immunology assays including enzyme-linked immunosorbent assay, fluorescence activated cell sorter, tissue culture, cell sorting, histology, and mouse handling, including injections. Send curriculum vitae and list of three references to: **Torrey Pines Institute for Molecular Studies, Attn: Human Resources, 3550 General Atomics Court, San Diego, CA 92121. Fax: 858-455-3796, e-mail: careers@tpims.org.** *Torrey Pines Institute for Molecular Studies is an Equal Opportunity Employer.*

POSTDOCTORAL POSITION with **Stuart Smith** at Children's Hospital Oakland Research Institute (**website: http://www.chori.org**) to characterize a system for fatty acid synthesis in human mitochondria (**Zhang et al.,** *J. Biol. Chem.* page 40067, 2003, and page 12422, 2005). Studies include: enzyme cloning and expression; reconstitution of the pathway in mitochondrial matrix extracts; assessment of the importance of the pathway to mitochondrial function using knockout approaches. Apply to **e-mail: ssmith@chori.org.** *Equal Opportunity Employer.*

University of Maryland Department of Kinesiology seeks a tenure-track **ASSISTANT PROFESSOR** in exercise physiology/molecular biology. Postdoctoral training required. Women and minorities especially encouraged to apply. See **website: http://www.hhp.umd.edu/KNES/** for information or contact: **James Hagberg, Ph.D., Department of Kinesiology, University of Maryland, College Park, MD 20742-2611. E-mail: hagberg@umd.edu.** *An Affirmative Action/Equal Opportunity Employer.*



Great scientists don't just fall from the sky.



Isaac Newton
1642–1727

Post your jobs on ScienceCareers.org with **Post and Go.**

- Jobs are posted within one business day and stay up for 8 weeks.
- Applicable jobs are also searchable on the following websites:
  - Biocompare
  - National Postdoctroral Association (NPA)
  - Stanford University School of Medicine
  - *Science*'s Signal Transduction Knowledge Environment (STKE)
  - *Science*'s Aging Knowledge Environment (SAGE)
- ScienceCareers.org averages over 1 million page views and over 75,000 unique visitors each month.[1]
- All jobs are included in our Job Alerts e-mail system.

All this exposure means you can find the right scientist for your vacancy quickly and inexpensively.

For more information,
contact Chris Normile
Phone: 202-326-6555
E-mail: cnormile@aaas.

ScienceCareers.org
We know science AAAS

1 *Science* Webtrends Reports.

POSITIONS OPEN

TENURE-TRACK ASSISTANT PROFESSOR IN CELL/MOLECULAR BIOLOGY
Oakland University
Department of Biological Sciences

The Department of Biological Sciences at Oakland University invites applications for a tenure-track Assistant Professor position to be filled by August 2006. We seek a candidate who will investigate the molecular mechanisms of eukaryotic inter- and intracellular signaling. A Ph.D. and postdoctoral experience are required as well as a strong research track record evidenced by publications. Laboratory space and competitive startup package will be provided. The successful candidate is expected to develop a vigorous, extramurally funded research program, to teach effectively at the undergraduate and graduate levels, and to mentor graduate students in doctoral programs.

The Department of Biological Sciences (**website: http://www2.oakland.edu/biology/**) is a modern, well-equipped, and research-oriented Department of 19 faculty members. Oakland University is a state-supported institution of 17,000 students situated on a beautiful 1,600-acre campus 25 miles north of Detroit.

Review of applications will begin on February 1, 2006, and continue until the position is filled. Applicants should submit curriculum vitae, statement of research plans and teaching philosophy, and key reprints and should have three letters of reference sent to:

Anne L. Hitt, Ph.D.
Search Committee Chair
Department of Biological Sciences
Oakland University
Rochester, MI 48309-4401
E-mail: biology1@oakland.edu

Oakland University is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.

ASSISTANT PROFESSOR
(tenure track, academic year)

The Medical Technology Program at Michigan State University seeks a team player with an earned doctorate in a relevant science to contribute to the teaching, scholarship, and service missions of the unit. Postdoctoral training or evidence of the ability to secure extramural funding is required. Professional laboratory certification (SCM, ABHI, NCA, ASCP, ABCC, or others) or clinical experience is highly desirable. Qualifications suitable to direct a medical laboratory and teaching experience are preferred. Teaching responsibilities will be appropriate to expertise. Review of applications begins January 3, 2006, and will continue until the position is filled. Send curriculum vitae and contact information for three references to: **Kathy Doig, Ph.D., C.L.S. (NCA), Medical Technology Program, 322 N. Kedzie Lab, East Lansing, MI 48824-1031. Telephone: 517-353-7800; fax: 517-432-2006; e-mail: doig@msu.edu.** *Michigan State University is an Affirmative Action/Equal Opportunity Institution.*

The Division of Plastic Surgery at Northwestern University Feinberg School of Medicine is accepting applications for the position of **RESEARCH ASSISTANT** or **ASSOCIATE PROFESSOR** to assume a position of leadership in an NIH-funded wound healing research laboratory. The person should have a background in animal model research and familiarity with a broad range of molecular biology technologies. Please contact:

Thomas A. Mustoe
Professor and Chief, Division of Plastic Surgery
Northwestern University
Feinberg School of Medicine
675 N. St. Clair Street – Suite 19-250
Chicago, IL 60611
Telephone: 312-695-6022
Fax: 312-695-5672
E-mail: tmustoe@northwestern.edu

POSITIONS OPEN

ASSISANT PROFESSOR PHYSIOLOGY/ANATOMY

The Department of Biological Sciences at San José State University seeks applicants for a tenure-track position at the level of Assistant Professor. Applicants must have a Ph.D. degree in the physiological or anatomical sciences and experience teaching courses in these disciplines. Applicants must demonstrate a potential for excellence in teaching and an ability to establish a research program involving undergraduates and M.S. graduate students. Research collaborations include opportunities at nearby biotechnology companies, Bay Area universities, Moss Landing Marine Laboratories, and NASA Ames Research Center. The successful candidate will participate in anatomy and physiology courses for nonmajors and may teach in courses for biology majors and graduate students. The new faculty member must address the needs of a student population diverse in age, ethnicity, and culture. For consideration send a letter of application, curriculum vitae, official university graduate transcripts, statement of teaching and research interests, and three letters of reference to: **Physiology and Anatomy Search Committee, Department of Biological Sciences, San José State University, One Washington Square, San José, CA 95192-0100.** Please include job requisition number JRN 012169 on all correspondence. Review of applications will commence immediately and continue until the position is filled. **Website: http://www.sjsu.edu/depts/Biology.** *SJSU is an Equal Opportunity/Affirmative Action Employer committed to the core values of inclusion, civility, and respect for each individual.*

POSTDOCTORAL FELLOWSHIP in G Protein Signaling and Regulation of Epithelial Cell Junctions. An NIH-sponsored Postdoctoral training grant position is available in the laboratory of **Dr. Bradley M. Denker** within the Renal Division at Brigham and Women's Hospital and Harvard Medical School in Boston to study the role of G proteins in regulating epithelial cell junctions. The goals of this work are to understand the signaling through G proteins that regulate the maintenance and assembly of the tight junction. Emphasis on two interactions (*JBC* **277:**24855, 2002 and *JBC* **279:**54983, 2004) will be the focus of this work. A strong background in molecular biology, biochemistry, and imaging is required as is a desire to learn and apply emerging methods. Applicants must have a Ph.D. and/or M.D. with no more than one to two years of postgraduate experience. Send curriculum vitae and three references to:

Bradley M. Denker, M.D.
Renal Division, Brigham and Women's Hospital
Harvard Institutes of Medicine
77 Avenue Louis Pasteur
Boston, MA 02115
E-mail: bdenker@rics.bwh.harvard.edu

POSTDOCTORAL POSITIONS are available immediately at The University of Utah in Salt Lake City to study the mechanisms of myofibrillar diseases linked to mitochondrial biogenesis and protein misfolding disorders. Outstanding candidates will be selected from major disciplines in molecular mechanisms of RNA regulation and biochemistry. Successful candidates will join an integrated team tackling the underlying mechanisms using short interfering RNA and conditional transgenic and gene knockout models. Please send curriculum vitae to:

Ivor Benjamin, M.D.
c/o Lori Kaumans
30 N. 1900 E., Room 4A100
Salt Lake City, UT 84132
E-mail: lori.kaumans@hsc.utah.edu

Selected references: **Christians E. et al.**, *Nature* **407:**693, 2000; **Yan et al.**, *EMBO J* 5164, 2002; **Christians and Benjamin,** *Meth. Enzym.* **35:**170, 2005; **Taylor and Benjamin,** *J Mol. Cell Card.* **38:**433, 2005.

POSITIONS OPEN

PLANT BIOLOGIST (PROGRAM DIRECTOR) AD-401-4 NATIONAL SCIENCE FOUNDATION
Division of Integrative Organismal Biology
Annual salary ranges from $88,369 to $137,713

The National Science Foundation's Directorate for Biological Sciences (BIO), Division of Integrative Organismal Biology (IOB) is seeking qualified candidates for one position as Program Director in plant biology. The Division (IOB) supports integrative research in emerging areas of plant biology including integrative plant biology, ecological and evolutionary plant physiology, plant-biotic interactions, and plant development. Individuals with extensive cyber experience are especially encouraged to apply. More information about IOB can be found on their **website: http://www.nsf.gov/div/index.jsp?div=IOB.** Appointment to this position is to be filled as a permanent Program Director. Applicants must possess a Ph.D. in plant biology or in an equivalent discipline. In addition, six or more years of successful research, research administration, or managerial experience beyond the Ph.D. are required.

The announcement E20060013-Permanent, which includes position requirements and application procedures, is located on NSF's Division of Human Resource Management **website: http://www.nsf.gov/about/career_opps/** or can be obtained by contacting **Myra Loyd** at **telephone: 703-292-4363.** Hearing impaired individuals may call **TDD 703-292-8044.** Applications must be received by December 6, 2005.

NSF is an Equal Opportunity Employer committed to employing a highly qualified staff that reflects the diversity of our nation.

POSTDOCTORAL POSITIONS in cellular and molecular immunology in a productive research environment: (1) to study the role of self-antigen processing and presentation in the induction of T cell tolerance and regulation of autoimmunity; experience in tissue culture and handling of mice/rats is required; and (2) to define unique markers of synovial vasculature and study their therapeutic applications in a rat model of arthritis; experience in molecular biology, particularly (T7) phage peptide display library, is essential. Fresh Ph.D. graduates preferred. Submit curriculum vitae and three letters of reference to: **Kamal D. Moudgil, M.D., Ph.D., Associate Professor, Department of Microbiology and Immunology, 660 W. Redwood Street, HH 323C, University of Maryland School of Medicine, Baltimore, MD 21201. E-mail: kmoud001@umaryland.edu; fax: 410-706-2129.**

POSTDOCTORAL PROJECTS in molecular biology and biochemistry are available at the FBI Laboratory in Quantico, Virginia. Projects involve development or evaluation of genotyping assays to analyze DNA from humans, microbial pathogens, and plants. Candidates should have a Ph.D. in genetics, molecular biology, biochemistry, or a related discipline. Experience with standard molecular biology techniques is desired. Familiarity with analytical instrumentation, RNA, short tandem repeat analysis, capillary electrophoresis, phylogenetics, and statistical analyses would be beneficial. Additional information may by found at **website: http://www.orau.gov/orise/edu/postdoc/pdneeds.htm.**

A **POSTDOCTORAL POSITION** is available to study a diverse gene family in the sea urchin that shows evidence of sequence "scrambling" and encodes putative immune proteins. A Ph.D. and expertise in molecular biology are required; bioinformatic analysis of genomes is a plus. E-mail a resume, list of publications, and names of three references to: **Dr. L. Courtney Smith, Department of Biological Sciences, 340 Lisner Hall, George Washington University, 2023 G Street N.W., Washington, DC 20052. E-mail**: **csmith@gwu.edu.** See **website: http://www.gwu.edu/~clade/faculty/smith** for more information.

TaKaRa

The Future of High Fidelity PCR

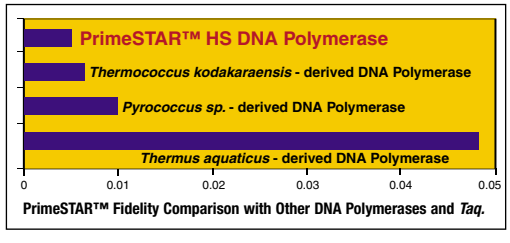
PrimeSTAR™

Takara Bio Introduces:

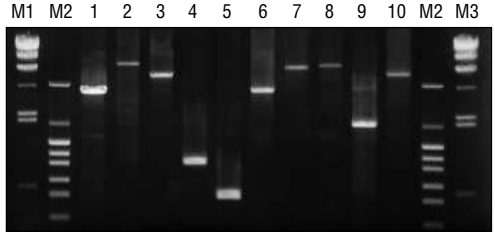
PrimeSTAR™ HS DNA Polymerase, a novel new high fidelity PCR enzyme which provides maximum fidelity as well as extended product length (8.5 kb for human genomic DNA; 22 kb for λ DNA). Targeted for demanding cloning (i.e. amplification of cDNA libraries) and sequencing applications, PrimeSTAR™ HS offers extremely high accuracy, and is the only enzyme whose fidelity is calculated by the method you use - sequence analysis. It also offers excellent amplification efficiency and shortened reaction times.

PrimeSTAR™ HS offers:

- **High Accuracy:** A strong exonuclease activity results in an extremely low error rate, with only 12 errors per 250,000 bp as determined by DNA sequence analysis.
- **High Efficiency:** Amplification efficiency higher than *Taq* Polymerase.
- **Robust Amplification:** Tolerance to varying reaction conditions means a single PCR cycling protocol can be used to amplify products of varying sizes.
- **GC-Rich Targets:** Robust performance even with GC-rich templates.



PrimeSTAR™ Fidelity Comparison with Other DNA Polymerases and *Taq*.



1. DCLRE1A 4 Kb
2. beta-globin 8.5 Kb
3. beta-globin 6 Kb
4. DCLRE1A 2 Kb
5. p53 0.5 Kb
6. p53 4 Kb
7. beta-globin 7.5 Kb
8. DCLRE1A 8 Kb
9. DCLRE1A 1 Kb
10. p53 6 Kb

M1: λ *Hind* III-digest
M2: pHY Marker

A Single PCR Cycling Protocol Produces Optimal Amplification of Fragments of Varying Sizes.

TAKARA BIO INC.
The Biotechnology Company™

Otsu, Shiga, Japan
Phone: +81 77-543-7247 Fax: +81 77-543-9254

USA: **Takara Mirus Bio Inc.** Phone: 888-251-6618 Fax: 608-441-2845
Europe: **Takara Bio Europe S.A.** Phone: +33 1 41 47 2370 Fax: +33 1 41 47 2371
Korea: **Takara Korea Biomedical Inc.** Phone: +82 31 739 3300 Fax: +82 31 739 3311
China: **Takara Biotechnology (Dalian) Co., Ltd.** Phone: +86 411 8764 1681 Fax: +86 411 8761 9946

For more information and a list of Takara distributors worldwide, please visit our website today!

www.takara-bio.com